

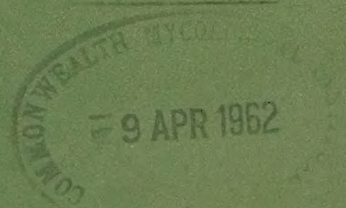
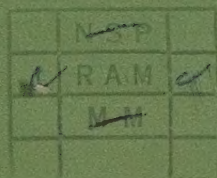
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Autotroph-heterotroph Relationships in Natural Waters

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Souhrn

Biologické zkoušky vod spolu s chemickou charakteristikou umožňují klasifikaci různých typů vod, ale jedno číslo pravidelně nestačí, abychom vodu správně hodnotili. Gabrielův biologický index stanovovaný at původní nebo modifikovanou metodou je cenné kritérium, jež nás může o stavu vody dobře informovat, jsou-li dodrženy nutné předpoklady; není to ovšem metoda a hodnota zcela kvantitativní.

Celková hodnota producentů, resp. fotosyntetisující hmota (autotrofové) proti celkové organické hmotě vůbec [hmota konsumentů plus reducentů (destruentů) plus rozpustěné organické látky] by tedy byla mnohem lepší mírou pro charakteristiku vody nežli prostý počet nestejnorodých jednotlivých individuí, i když je doplněn přídatnými indexy.

Proto lepší představu o stavu biomasy poskytuje srovnání obsahu chlorofylu (jako charakteristika fotosyntetisující hmoty) s obsahem „organického uhlíku“. Dosavadní běžnou konvenční metodu biologické spotřeby kyslíku je nutno doplnit standardizovanou obdobně konvenční metodou biologické produkce kyslíku.

Literatura uvádí velmi mnoho různých způsobů, jak stanovit „organický uhlík“ a chlorofyl; významnější z nich byly kriticky a experimentálně zhodnoceny v práci ŠESTÁKOVĚ (1955, 1958a, b). Z řady vyzkoušených modifikací se nejlépe osvědčily postupy navržené v textu. Poměr producentů a konsumentů s destruenty je nutno v praxi vždy zjednodušit, musíme se však vždy snažit o to, aby byl vyjádřen co nejpřesněji. Nelze se spokojit číslem nebo rovnicí, nýbrž je nutno vždy podle možnosti respektovat i další faktory, jako jsou např. složení společenstev, vztahy allelobiologické, antibiosa a specifické poměry různých lokalit; je tedy nutno respektovat nejen kvantitativní hodnoty, nýbrž hodnotit i kvalitativní charakter a vztahy různých organismů.

Summary

Biological tests of waters combined with determinations of their chemical nature make it possible to classify different types of waters, but usually one figure does not suffice for a correct evaluation of a given water. GABRIEL'S biological index is a valuable criterion which can give us a good deal of information about the state of the water if the necessary conditions are adhered to; it is not, however, a completely quantitative method and value.

The total body of producers, that is photosynthesizing matter (autotrophs) compared with the total organic matter as a whole [the body of consumers plus reducers (destroyers) plus dissolved organic matter] would be a much better criterion for characterizing the waters than a simple count of various individual bodies, even if this were to be supplemented by the relevant indices.

The usual conventional method, employed hitherto, of a biological oxygen demand must be supplemented by a similarly standardized conventional method of biological production of oxygen.

The literature on the subject gives many different methods for establishing "organic carbon" and chlorophyll; the most important of these have been evaluated critically and experimentally by ŠESTÁK (1955, 1958a, 1958b). Of a number of tested modifications the procedures outlined in this article proved to be the best. The relationship between producers and consumers plus destroyers must always be simplified. We cannot content ourselves with a figure or an equation, but must respect other factors as far as possible.

The evaluation of different waters has for long been the fundamental problem not only in hydrobiology (biological productivity of waters), but also in the field of health services (hygiene, the supply of drinking and other water, spas), agriculture (irrigation) and industry (supply of water for production processes). For practical purposes an easy and simple method is needed, which can provide as rapidly as circumstances permit, a quantitative basis for the evaluation of waters. Chemical and biological tests must, at the same time, complement each other (PRÁT 1947, 1956). The usual methods are described in detail or summarized in various papers; we give references here only to some of the latest works (BULÍČEK 1957; CYRUS and CYRUS 1950; HANUŠKA et al. 1956; JAM 1953; LAPTEV 1955; RAZUMOV 1957; STARMACH 1952, 1955, 1956; SYMON 1948; SYMON et al. 1954; TRNKA et al. 1957; ŽDANOV and RJABOVA 1956; earlier references, not given here, are contained in the papers mentioned).

Biological tests of water are sometimes unfavourably criticized, not for their actual nature, but on account of incorrect procedure in carrying them out (LUTONSKÝ 1956; ŠVEC 1956). It is not, of course, necessary to discuss the fact that careless work can ruin even the most perfect method. It is not, however, possible to agree with the contention that biological tests reflect only the momentary state of water at the time of sampling (ŠVEC 1956, p. 160).

With the exception of cases where there is a rapid and strong external influence (e. g. a sudden inflow in running water or sudden artificial intoxication), biological tests in fact provide evidence of equilibrium attained over periods of varying length before samples are taken; a biological test can show this previous state, which is sometimes different from the momentary, chemically determined state of water (cf. e. g. PRÁT 1947, 1952, 1956).

Many different kinds of biological tests can be used. They are usually combined with chemical and physical data. In many cases productivity is given in terms of photosynthetic assimilation. Analyses of the amount of chlorophyll in water can throw light on a number of questions, but only from one point of view; it is necessary to know not only the capacity of organisms for increasing oxygen production (thereby at the same time, of course, the increase of assimilates), but also the conditions of oxygen consumption, or the amount of oxidizable substances in water. Attempts to use chlorophyll for the determination of the amount of phytoplankton are of an earlier date (for references cf. ŠESTÁK 1955).

The question of establishing types of waters according to their purity, the organisms present and productivity has not as yet been satisfactorily solved. The complexity of these questions is shown in the reports and discussion at the XIII. International Limnological Congress (e. g. ELSTER 1958; HRBÁČEK 1958; OHLE 1958) and in a number of articles in recent volumes of the journal *Limnology and Oceanography* (e. g. OHLE 1956).

For a correct evaluation of the properties of water we must know not only the chlorophyll content, but also the mutual relations between producers, reducers and consumers; their numerical expression was introduced by GABRIEL (1940, 1943, 1946, 1954) as the so-called "biological index". This is based on the counting of centrifuged plankton under the microscope. It is intended to provide information about the self-purifying capacity of the water concerned.

Gabriel's biological index, established either by the original or by a modified method (LUTONSKÝ 1956) is a valuable criterion, which can give us a good deal of information about the state of water if the necessary conditions are adhered to; it is not, however, a completely quantitative method and value. Some of its drawbacks have been pointed out by CABEJSZEKOVA and MALANOWSKI (1955), STANISLAWSKA (1955), TUBOROYSKI (1957).

The principle of the method—the counting of producers, consumers and reducers and the recording of their mutual relationship

$$\left(\frac{2 \times \text{number of producers}}{\text{No. of reducers} + \text{No. of consumers}} \right)$$

—is certainly correct. In practice, however, it is not possible to count individual bacteria or to evaluate zooglae of different sizes; this is the most serious obstacle in the case of reducers or destroyers. New opportunities are offered by the method of determining bacterial mass by means of membrane filters, which is being steadily perfected (POKORNÝ 1957), but it is not easy to incorporate its results into Gabriel's index.

Different algae, e. g. *Chlorella*, *Scenedesmus*, *Eudorina*, *Pediastrum*, *Euglena*, etc., produce very different effects according to their size and photosynthesis intensity, and this applies both when individual cells or colonies are counted. Consumers also show considerable differences in size. Quantitative results are not even given by estimates of biomass volume according to various models (e. g. LOHMANN 1908).

The counting method can only take into account formed particles. Even if the so-called amorphous parts were to be included in the formula of the index (HANUŠKA 1949) the method remains very inaccurate and arbitrary. This is also due to the fact that we cannot distinguish inorganic particles from organic under the microscope without recourse to complicated micro-analysis. Coagulates of bi-valent and tri-valent iron behave very differently in water, but in counting we have to evaluate them equally.

Substances dissolved in water can sometimes, however, be of greater importance for the evaluation of water than the seston. Whatever view may be taken of PÜTTER's theory of dissolved nutrients of water animals, it is not possible to ignore the organic substances dissolved in water. The total content of unformed, dissolved organic substances in water varies considerably and can be quite high. As early as 1942 JUDAY (1942) reported from some lakes in Wisconsin that the amount of dissolved substances in water may exceed the amount of organic matter of water fauna and flora. The relation of the quantity of plankton to the amount of dissolved organic matter was reviewed by SAUNDERS (1957).

Recently LEWIN (1956) has described *Chlamydomonas mexicana*, which can set free in the medium 20 to 25% of its total production of organic matter in the form of dissolved polysaccharides. In the case of the species *Chl. parvula* and *Ch. peterfii*, deliquescent mucus forms 40 to 60% of the total production of organic matter and in older cultures it considerably increases the viscosity of the environment. In the light of these findings and of the literature on the subject LEWIN points out the consequences for plankton succession and for the growth of heterotrophic bacteria, the possibility of forming chelate compounds with metals, and the influence of hydrocolloids on soil properties. SHAPIRO (1957, p. 170) reports far more dissolved organic matter than seston for Linsley Pond. It is, therefore, important that these dissolved substances should be taken into consideration in the classification of waters.

The total body of producers, that is photosynthesizing matter (autotrophs) compared with the total organic matter as a whole [the body of producers plus consumers plus reducers (destroyers) plus dissolved organic matter] would be a much better criterion for characterizing the waters than a simple count of various individual bodies.

A better idea of the state of the biomass-production is, therefore, obtained by means of a comparison of the chlorophyll content (as an indication of photosynthesizing matter) with the "organic carbon" content. This procedure was adopted by VINBERG and ZACHARENKO (1950), but they stated the correlation coefficient only for a limited range of conditions of plankton. REY (1952) suggested the comparison of chlorophyll with protein content. HOGETSU and ICHIMURA (1954) determined the relation of chlorophyll to organic carbon in seston from various depths.

Although none of these experiments has led to definite conclusions they support the view that the relation between the amount of chlorophyll and the amount of organic substances (living, organized and dissolved) can provide a valuable criterion for the estimation of the properties of water.

The calculation of the relation between the amount of chlorophyll and the total amount of organic matter (determined from dry weight or by carbon content) would thus give a better indication of the interrelation of autotroph and heterotroph activity than counting of organisms. Insofar as there is a sufficient amount of plankton or organic matter in a sample it is only necessary to evaporate the water and determine dry weight and deduct the amount of ash. However, some organic substances may be decomposed during

evaporation, thus giving rise to unnecessary experimental errors. For this reason (particularly when there is only a small quantity of organic matter available) it is better to determine the amount by chemical means.

The combustion of organic substances and the determination of freed carbon dioxide by elementary analysis is a relatively complicated and lengthy process; with rising demands on accuracy hydrobiology does indeed require ever more and more complicated laboratory methods (e. g. KAY 1954a, 1954b), but in practice it is usual to use methods of wet oxidizing of carbon, even though the results are not so reliable (*cf.* ŠESTÁK 1955, 1958b). Using these methods we always obtain a lower value for so-called organic carbon, i. e. oxidizable substances, and, which is important, a value which varies according to the chemical nature of the substances. This disadvantage can be compensated by the fact that in hydrobiology we are usually more concerned with the amount of substances capable of oxidation under natural (milder) conditions, with the consumption of oxygen, than with the total amount of all organic matter (including that which is not readily oxidized). On the other hand, however, it is not only the total amount of oxidizable substances and their capacity for oxidation which is important, but also their quality. Thus, for example, humus substances (humic acids) are burned with difficulty and when oxidation conditions are mild, amount of humus is only partially determined (ŠESTÁK 1955, 1958b; SHAPIRO 1957, p. 171); but it is just these compounds which are of considerable importance for the development of organisms, whether positively (PRÁT 1955, 1956) or negatively (dystrophic waters), and they are, therefore, biologically very important.

In practice, however, a simplified conventional method, which gives results that can be easily reproduced, must suffice. If a certain method is uniformly employed it is at least possible to obtain relative results which are comparable.

In order to determine the total amount of oxidizable substances, so-called organic carbon (i. e. carbon in living organisms, carbon in non-living organisms but in an organized state and unorganized dissolved organic compounds), a sample of water is taken direct for analysis. Where it is necessary to supplement and differentiate these values the carbon is determined in the residue on the filter or in the centrifugate (of seston) and dissolved substances in the filtered or centrifuged water.

Many different methods of determining "organic carbon" and chlorophyll are given in the literature; the more important of them have been critically and experimentally reviewed by ŠESTÁK (1955, 1958a, b). Of a number of tested modifications the following procedures have proved the best:

A. Determination of organic carbon

1. Bichromate method for samples with at least 20 mg. C/l:

To 5 ml. of water sample in a thick-walled test-tube SIAL (or PYREX), size 30 × 160 mm., are added 5 ml. of 0.5 N $K_2Cr_2O_7$ solution in conc. H_2SO_4 and the tube is heated for 60 min. in a steam bath in a covered vessel. The contents of the test-tube is then poured into a measuring flask, the volume is made up to 250 ml. and 1 hour after dilution 3 portions of 50 ml. are taken from the sample. To each is added 1.5 ml. of 20% aqueous solution of KI and this is titrated with 0.05 N or 0.025 N solution of $Na_2S_2O_3$ against a starch indicator until the colour disappears. In the given portion 1 ml. of 0.05 N $Na_2S_2O_3$ corresponds to 0.15 mg. of carbon. The average from parallel portions is converted to correspond to 100 ml. of water (multiplication by 100).

It may be mentioned that a similar bichromate procedure was included among the standard methods in the latest edition of the well-known American manual (Standard methods . . . , 1955). It differs from the method recommended above particularly in the lower resultant concentration of bichromate in the mixture during the combustion of the sample (0.04 N), which lasts for 2 hours under reflux, and in the final titration with ferrous-ammonium sulphate using ferroin indicator. For waters containing substances difficult to oxidize, catalysis with silver ions is recommended (this did not prove successful, ŠESTÁK 1955, 1958b). If Ag_2SO_4 is not used for

catalysis it is necessary to deduct a correction for chlorides ($\text{mg. Cl/1} \times 0.23$). It is also necessary to take into account the possibility of oxidation of iron compounds.

2. The permanganate method according to KUBEL for samples up to 10 mg. C/1.

In a 500 ml. Erlenmeyer flask with a special tube for calming boiling, 5 ml. of 25% H_2SO_4 by volume are added to 100 ml. of the water sample. As soon as the mixture boils, 15 ml. of 0.01 N KMnO_4 are added and the resulting mixture is kept at the boiling point for a further 10 min. Then 15 ml. of 0.01 N oxalic acid are added and the liquid retitrated with 0.01 N permanganate solution until the first pink colour appears. In retitration 1 ml. of 0.01 N KMnO_4 solution corresponds to 0.03 mg. of carbon oxidized in the sample.

"Carbon" as determined by the manganistan method is usually one-fourth to one-fifth (or even less) of the carbon found by the biochromate method and it is necessary to take this into account in calculation.

B. Determination of pigment content (chlorophyll).

A layer 1 to 1.5 cm. thick of glass sand (grains smaller than 100 μ , average size of grains 12 to 25 μ) is placed on filter paper in a small Büchner funnel and the sample is filtered under suction. The sand with plankton is then poured into a mortar and ground for about 3 min. until coloured stripes are no longer formed; it is then returned to the funnel and extracted with 5 ml. of non-aqueous acetone, then 85% acetone, finally non-aqueous acetone again. The extract is refiltered through the Jena sinter-glass No. G4, made up to 20 ml., measured on a photometer. Extinction is compared with the standard curve and converted in terms of percentage of chlorophyll (pigments) in relation to the organic matter in the sample. Examples of chlorophyll determination are given e. g. by SMITH and BENITEZ (1955), calculation nomographs by DUXBURY and YENTSCH (1956).

Using the methods described above, the relation of "organic carbon" and chlorophyll in samples of algae grown from cultures of the Collection of Autotrophic Organisms of the Czechoslovak Academy of Sciences (PRÁT 1948) was studied. In the survey given in two tables the values obtained by counting cells in a counting chamber have been added for comparison.

There is ample evidence in literature on the subject and in the facts mentioned above that a number of difficulties are met with during the determination

Table 1. *Chlorella vulgaris* var. *viridis* CHODAT. Comparison of results obtained by various methods (average of 2 to 6 determinations). Amount of chlorophyll expressed in extinctions of 10 ml. acetone extract, measured by means of Pulfrich photometer in a 5 cm.-long glass absorption cell. Average concentration of algae 1, 1/2, 1/3, 1/4 and 1/6. Values for diluted samples are given below as percentages.

Concentration	Counting in chamber		Organic carbon				Chlorophyll acc. to S 61 in 5 ml. of sample (method B)	
			KMnO ₄ (A 2)		K ₂ Cr ₂ O ₇ (A 1)			
	No. of cells in 5 ml.	%	mg. C in 5 ml.	%	mg. C in 5 ml.	%	extinction	%
1	11,000,000	100	0.112	100	0.637	100	0.450	100
1/2	5,000,000	45.5	0.054	48.3	0.262	41.2	0.260	57.8
1/3	3,480,000	31.6	0.032	28.6	0.206	32.3	0.150	33.3
1/4	3,300,000	30	0.030	26.7	0.169	26.5	0.096	21.3
1/6	2,240,000	20.3	0.017	13.6	0.075	11.8	—	—

Table 2. Amount of pigments (in extinctions $\times 10^3$) from measurements with filters S 61 and S 47, corresponding to 0.1 mg. C (by bichromate method) and to 1 million algae cells (from count in chamber).

Pigments determined by method B, only for *Chlamydomonas* cultures using 3 times repeated extraction from filters giving lower values (ŠESTÁK 1958a). Pigments measured in acetone extract of 10 ml. volume by means of Pulfrich photometer with 5 cm.-long glass absorption cell. Both cultures of *Scenedesmus* were about 3 months old, grown with the same illumination in liquid medium L 41 (PRÁT 1948). To medium of culture I added soil decoct, which in culture II was replaced by A—Z solution. The difference in chlorophyll content for cultures I and II is significant ($\bar{d} = 5.6$, $t_{11} = 4.78$, $P < 0.001$).

Algal species	Filter S 61		Filter S 47	
	amount of chlorophyll corresponding to			
	0.1 mg. C	1 mil. cells	0.1 mg. C	1 mil. cells
<i>Chlamydomonas gyrus</i>	56	382	231	1390
<i>Chlorella vulgaris</i> var. <i>viridis</i>	71	41	229	133
<i>Scenedesmus obliquus</i> cult. I	47	30.6	164	105
<i>Scenedesmus obliquus</i> cult. II	—	36.2	—	125

of the relation between “organic carbon” and chlorophyll, such as: the percentage content of chlorophyll is not constant even within one algal species, it depends on nutrition, on the amount of ions and organic substances in the environment, on the age of the culture and on other factors; individual groups of algae contain photosynthetic pigments of varying activity (cf., e. g., BLINKS 1955). Photosynthesis is not a linear function of chlorophyll content. Even dead cells continue for a long time to contain some non-decomposed chlorophyll. In view of this the only solution would be to determine photosynthetic intensity directly, or the amount of freed oxygen. As early as 1947 PRÁT pointed out that the existing method of biological oxygen demand would have to be supplemented by the value of biological oxygen production not only using “light” and “dark” bottles in the natural surroundings, but determining photosynthesis in definite quantitatively characterized conditions. While, however, the BOD method is only an agreed test and its different procedures are still under discussion (dilution water, degree of dilution, period of exposure, unnatural experimental conditions), in the determination of photosynthesis a further technically difficult problem arises, i. e. defined, constant and easily reproducible illumination. So long as an acceptable and uncomplicated solution of this has not been found these experiments may serve as a qualitative criterion, but they cannot offer a quantitative evaluation comparable for different experiments. However, even positive or negative results of tests in the light (increase or decrease of oxygen) can give valuable information for different waters.

In the meantime the relation of organic substances (“organic carbon”) in water to the amount of chlorophyll provides at least an approximate value, which can give useful information about the activity of autotrophs and heterotrophs.

If the ratio of chlorophyll to the total organic matter (organized and dissolved) is high, a prevalence of photosynthesis is indicated, that is a high content of producers. If on the other hand the ratio of chlorophyll to total organic matter is low, reducers, destroyers and consumers (or in some cases dissolved organic substances) preponderate. In waters lacking in producers the chlorophyll content drops to nought; in this case the absolute value of organic substances is decisive for the nature of the water.

This means that if there is 2 to 4% of chlorophyll in the dry weight (i. e. about 4 to 8% with regard to the total amount of "carbon"), a high proportion of autotrophs, producers, can be assumed. The lower the chlorophyll content the greater the preponderance of heterotrophs.

This conception is, however, quite schematic and in definite cases it is necessary to evaluate not only the figures obtained, but also to take into account the forms of organic matter and the kinds of organisms they represent. J. HRBÁČEK has called our attention to cases where assimilating phytoplankton is rapidly consumed by strongly reproducing *Daphnia* (grazing activity of the zooplankton). Under such conditions a low or non-existent amount of chlorophyll would be found with a large amount of organic substances (living individuals). These figures would provide incorrect information about the properties of the water. It must be repeated that figures are only an aid, which make it possible to record certain conditions in a quantitative sense, but not absolutely. In every case it is necessary to consider as far as possible all the circumstances in order to give numerical values their true significance. Further, in addition to the quantity the quality of organic substances must be stated, that is they must at least be typified according to the fraction as dissolved (in the filtrate) and organized and these should again be divided at least into living and dead. What is more, this quantitative result must be described qualitatively, for it is impossible to regard a living mass of *Infusoria*, *Rotifera* and *Crustacea* or even molluscs and fish as equivalent to each other. The important part played by fish breeding in influencing the character of reservoirs has been emphasized by HRBÁČEK (1958).

The total nitrogen content could also be taken as a basis for comparison; it would correspond more closely to the living organisms. However, the nitrogen content of organisms shows a much wider range of variation than does carbon content; nitrogen compounds are often difficult to oxidize (ŠESTÁK 1955). Therefore, while in some cases nitrogen content can serve as a suitable supplementary basis, as a general rule it is even less suitable than the amount of oxidizable carbon substances.

The method here suggested is not intended as a substitute for Gabriel's biological index nor for any other form of evaluation, but as a supplement. So long as some other easy and more accurate method has not been worked out Gabriel's biological index remains an important aid for the evaluation of waters.

In hydrobiological literature there is frequent mention of frequency (numerousness) and of production or of weight of the biomass. Gabriel's index determines frequency relations, the method concerned with the relations of organic substances and pigments approaches nearer to actual production.

It has already been emphasized while describing methods that the oxidation methods for the determination of so-called total organic carbon do not give completely quantitative results, that the values obtained can be compared only if a uniform method is used and that they must be regarded as relative. There are also a number of other factors which must always be considered in the assessment of water. It is very difficult to fix the share of chemosynthesis in this complex; although it is not great (KUZNETSOV, 1958), it has to be reckoned with.

With regard to the relation between producers and consumers it has been the custom so far to conceive of it as the preponderance of assimilation (photosynthesis) over dissimilation. But even granting their fundamental significance it is not possible to fit these relations into such a simplified scheme. No equation can be made to fit the influence of one organism on another,

neither allelobiological relations nor the mutual influence of individuals of the same species. This is the case within groups of producers and consumers. Various interspecific relations can act not only on their growth, but also on their activity (POSPÍŠIL 1952). A few notable examples will suffice to illustrate the mutual influence of organisms. As early as 1914 PRÁT (1914) wrote: "Among only slightly developed filaments of algae (*Cladophora*, *Spirogyra*) in a dish there appeared *Saprolegnia* on a mosquito larva. In order to maintain its growth I put dead flies into the dish and after a time I observed that unicellular green algae had appeared on the bodies of the flies among the *Saprolegnia*. The former appeared to be doing well, while *Saprolegnia* stopped growing and finally disappeared. New flies, which were later introduced into the dish, did not become infected with *Saprolegnia*, similarly *Saprolegnia* did not appear on flies put into water from the original culture which had been poured into another dish". — Today the literature on antibiotic influences is voluminous, but relatively little of the work applies to natural conditions in the field (STEEMAN-NIELSEN 1955). With regard to the relations between autotrophs and micro-organisms the work of MENCL (1956a, 1956b) may be mentioned; even if his results are not completely approved, they show the importance of this problem and its significance in relation to the new approach to the assessment of water. Quite a number of cases of symbiosis of autotrophs and micro-organisms are known. The varying mutual influence of different components of plankton and in particular of water flora have often been described (FORT 1956; KOMÁREK 1955). GORYUNOVA (1955) describes cases, which she refers to as хищничество (robbery) of blue-green algae.

All these interrelations are undoubtedly of great importance in the general balance of biocoenosis, but they cannot be expressed by any equation. We must, however, take them into account.

Although it will now be clear that the relation between producers and consumers plus reducers (destroyers) has always to be simplified in practice. it is not possible to omit this value, rather it is necessary to try to express it as accurately as possible and to take into account other factors as well, as far as circumstances permit.

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К вопросу о взаимоотношениях автотрофов и гетеротрофов в водах

СИЛВЕСТР ПРАТ и ЗДЕНЕК ШЕСТАК

Резюме

На основе многих литературных данных обсуждается потребность использования биологических и химических тестов в классификации разных типов вод. В качестве дополнения тнз. биологического индекса Габриеля

$$\left(\frac{2 \times \text{количество продуцентов}}{\text{количество редуцентов} + \text{количество консументов}} \right).$$

устанавливаемого подсчетом планктонных организмов, рекомендуется сравнение содержания хлорофилла с содержанием органического углерода в данной воде. Для этого рекомендуются методы (определение органического углерода окислением перманганатом или бихроматом, колориметрическое определение хлорофилла), которые экспериментально изучались и оценивались. Обычный метод BOI надо дополнить стандартизированным биологическим методом продукции кислорода.

The Cytomorphological Effects of Low Temperatures Studied by Tempered Fixatives

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Souhrn

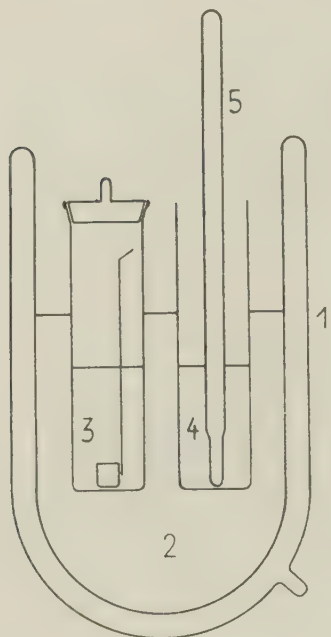
V této práci je sledován mechanismus cytomorfologického působení nízkých teplot na listy 4 dny naklíčené pšenice. 1. Odstrížené špičky listů byly ponořeny v pletivovém košíčku do tekutého dusíku a bez rozmrznutí co nejrychleji nafixovány. 2. Stejným způsobem byly zpracovány další objekty, avšak po vyjmutí z tekutého dusíku byly ponechány volně na vzduchu rozmrznout a teprve pak nafixovány. 3. Další objekty byly nafixovány přímo, tj. bez zmrazení tekutým dusíkem. Fixáže byly vytemperovány na -76° , -27° , $+27^{\circ}$ a $+56^{\circ}$ C. Fixace v Carnoyově alkoholotové směsi 20 min, v Lisonově směsi 2 hod. Odvodnění a zalití bylo provedeno na vibrátoru. Řezy byly barveny kyselým fuchsinem podle Drawerta a Regaudovým železitým haematoxylinem. Výsledky uvádí tabulka (popsány chloroplasty špičky prvního zeleného listu).

	-76° C	-27° C	$+27^{\circ}$ C	$+56^{\circ}$ C
Z tekutého dusíku přímo do fixáže	normální	normální	nepravidelné útvary a normální	nepravidelné útvary a normální
Z tekutého dusíku 10 min. rozmrznout, pak do fixáže	nepravidelné útvary	nepravidelné útvary	nepravidelné útvary	nepravidelné útvary
Přímo do fixáže	deformované a normální	deformované a normální	normální	normální

Stejným způsobem zmrazené, avšak různě rozmrznuté objekty dávají odlišné výsledky. Z toho vyplývá, že k destrukci chloroplastů dochází při rozmrazání tkáně. Je však nutno připustit, že v případech, kdy zmrazení je pomalé, pracuje-li se za vyšší teploty (asi -20° C), anebo je-li objekt příliš veliký, mohlo by dojít k destrukci při zmrazování. Destrukce chloroplastů nenastává současně s destrukcí jádra, ani nemusí být destruovány všechny chloroplasty současně.

Summary

The mechanism of the cytomorphological effects of low temperatures was studied by the fixation of young leaves of *Triticum vulgare* with tempered alcoholic fixatives. Some specimens were plunged into liquid nitrogen, allowed to thaw and fixed; other objects were plunged into liquid nitrogen and fixed without thawing; control objects were fixed directly. At various temperatures of fixatives (-76 , -27 , $+27$, $+58^{\circ}\text{C}$) different results were observed on the objects pretreated in the same manner. It was thus demonstrated that the destruction processes occur, in this case, during thawing.



Scheme 1. The arrangement of baths for tempering of fixatives. 1 — Dewar flask, 2 — Water bath or cooling mixture, 3 — Alcoholic fixative, 4 — Ethanol 96%, 5 — Thermometer.

Introduction

The effect of temperature on fixation processes has been very little studied hitherto (e. g. fixation of mitochondria by Regaud's fluid in a refrigerator, fixation of glycogen by Lison's fluid, cold acetone in histochemical procedures etc.). Having developed the technique of cutting in a frozen state, various methods of handling frozen sections were studied (SOLGER 1926). New attempts appeared when freezing—drying and freezing—substitution were developed (BELL 1956). Starting with the results of GENÈVES (1955) and the author (1958), cytomorphological effects of low temperatures with tempered alcoholic fixatives have been studied in this work.

Material and Methods

Leaf tips of wheat, *Triticum vulgare* VILL. (Stupická vouska), germinated for 4 days in Petri dishes on filter paper moistened by tap water, at $+22^{\circ}\text{C}$, with 12 hours illumination, were used. The height of shoots was 2—3 cm. The tips (coleoptile + 1st green leaf) 1 cm. long were cut off, placed in metallic gauze baskets (diameter and height of each 1 cm.) and stoppered with wool.

The specimens were then prepared in three ways:

1. The specimens were submerged in liquid nitrogen for 3 min, then directly transferred (without thawing) into tempered fixatives.
2. Other specimens were submerged in liquid nitrogen for 3 min, after removal the baskets with objects were placed for 10 min. on a table, having been allowed to thaw first and then fixed with the tempered fluids.
3. Control specimens were fixed with the tempered fluids without preliminary freezing.

For tempering of fixatives water baths were used for temperatures of $+58$ and $+27^{\circ}\text{C}$, mixtures of CCl_4 + solid CO_2 for -27°C and $\text{C}_2\text{H}_5\text{OH}$ + solid CO_2 for -76°C in one litre Dewar flasks. Fixation for 2 hours at $+58^{\circ}\text{C}$ was carried out in a water bath with automatic regulation of temperature. For arrangement see scheme 1. The temperature varied $\pm 2^{\circ}\text{C}$ from average values. The fixatives, always 20 ml. in well-stoppered bottles, were freshly prepared and tempered 30 min. before use (Carnoy: ethanol absol. 3 parts + glacial acetic acid 1 part, fixation for 20 min. Lison: saturated solution of picric acid in ethanol 96% 85 parts + formalin 10 parts + glacial acetic acid 1 part, fixation for 2 hours). After fixation the specimens were placed for 10 min. in ethanol absol. I at laboratory temperature ($+20^{\circ}\text{C}$) being removed from the metallic baskets. Dehydration and clearing was done on a shaker (c.f. HRŠEL 1952): ethanol absol. II. 30 min., III. 30 min., ethanol—benzene 30 min., benzene I. 30 min., benzene II. 30 min., benzene—paraffin at 37°C 30 min. and embedding in pure paraffin overnight at $+56^{\circ}\text{C}$. When mounted, the longitudinal sections $4\ \mu$ were deparaffinised and stained with buffered acid fuchsin (DRAWERT 1936/7): 0.1% solution of Acid Fuchsin S (Lachema) in HCl —phosphate buffer pH 1.25, 30 min., ethanol 70% 30 sec., ethanol 96% 30 sec., isopropanol I. 1 min., II. 3 min., xylene I. 1 min., II. 3 min. or with Regaud's haematoxylin.

Results

The chloroplasts in the tips [of young wheat leaves were studied.

	-76°C	-27°C	$+27^{\circ}\text{C}$	$+58^{\circ}\text{C}$
From liquid nitrogen directly to fixative	Normal	Normal	Irregular formations and normal	Irregular formations and normal
From liquid nitrogen fixed after 10 min. thawing	Irregular formations	Irregular formations	Irregular formations	Irregular formations
Directly to fixative	Deformed and normal	Deformed and normal	Normal	Normal

“Irregular formations” are clumps of material, probably from destroyed chloroplasts. The borders of “deformed chloroplasts” are irregularly shaped and they are sometimes stuck together.

During temperation, some changes in the composition of fixatives occur (darkening of Lison's fluid at $+58^{\circ}\text{C}$, partial freezing of Carnoy's fluid at -76°C), but the table of results demonstrates that these processes are not responsible for the cytomorphological changes observed.

Discussion

The cytomorphological method is one of the techniques for studying cold death. We do not know the mechanism of cold injury (SMITH 1954, MERYMAN 1956, 1957). In interpreting our results, we must have in mind the velocity of the thermal changes, the minimum temperature reached, the time of exposure to low temperatures, the phase reversal, etc. We must distinguish the processes during freezing and during thawing. For this reason many works

are not comparable (BUGAEVSKY 1955, 1957, LEVITT 1957). I hope, the work of GENÈVES and the present paper are to some extent comparable.

The statements of GENÈVES that the specimens undergo the cytomorphological changes which are observed after plunging into liquid air, when being frozen and not when thawed, and that plunging into liquid air is an unsuitable technique of fixation, are not supported by the present work. In the present work the results obtained with chloroplasts of wheat leaves support the conclusion that when the specimens have been frozen by plunging them into liquid nitrogen the cytomorphological changes occur during thawing. This statement is supported by the experiences of many authors working with freezing—drying and freezing—substitution (authors's results in this field c.f. BENEŠ and HRŠEL 1958).

Other cases are to be distinguished when the specimens are frozen slowly, to a higher temperature (-20°C) and when the specimen is large. In this case the changes may occur during the freezing.

In the present work, destroyed chloroplasts were observed with a normal nucleus in the same cell. Normal and destroyed chloroplasts were also found together in the same tissue. The two following problems have been touched on here:

1. Are all of the cellular constituents of any kind (chloroplasts) in the same tissue altered by cold simultaneously?

2. Is the mechanism and time of cold destruction the same for various kinds of cell constituents (chloroplasts, nucleus)?

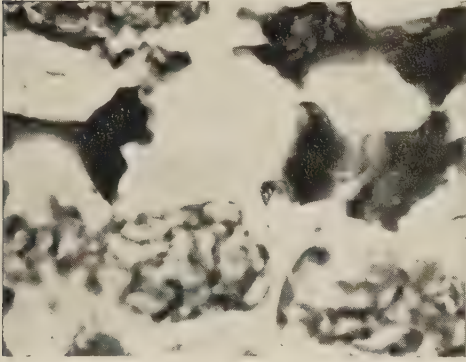
A comparison of cytological and biochemical data would be very interesting in connection with the problem of the preservation of active chloroplasts in a frozen state (SMITH et BENITEZ 1954, BISHOP et al. 1955, CLENDENNING et al. 1956). Some cytological studies were made in connection with diagnosis of frost resistance (GENKEL and OKNINA 1954).

References

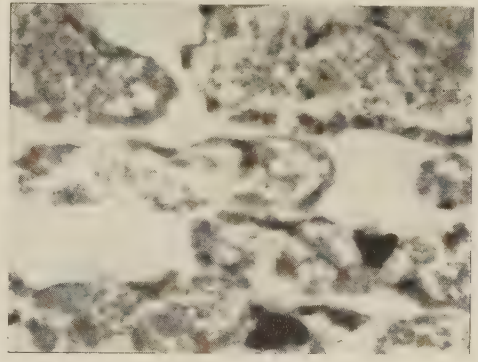
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THE CYTOMORPHOLOGICAL EFFECTS OF LOW TEMPERATURES

Triticum vulgare VILL., chloroplasts in leaf cells, fixation with tempered Carnoy's acetic alcohol, staining with buffered acid fuchsin.

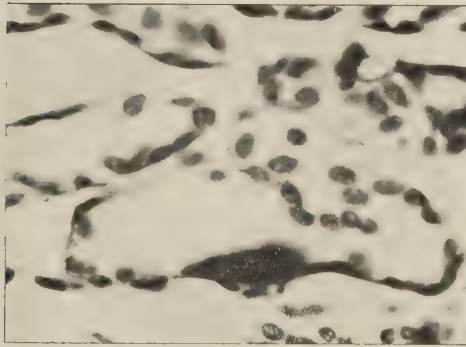


1a.

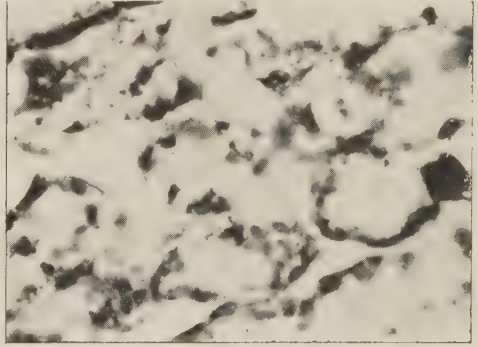


1b.

Fig. 1a, b. After plunging into liquid nitrogen 10 min. thawed and then fixed with Carnoy at a) -27°C , b) $+27^{\circ}\text{C}$.

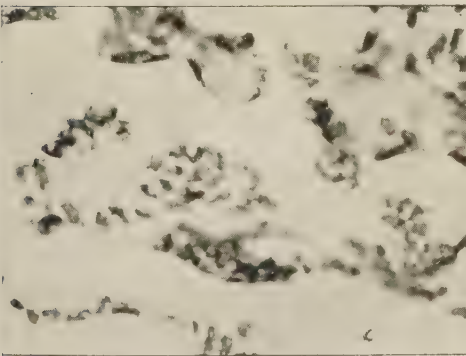


2a.

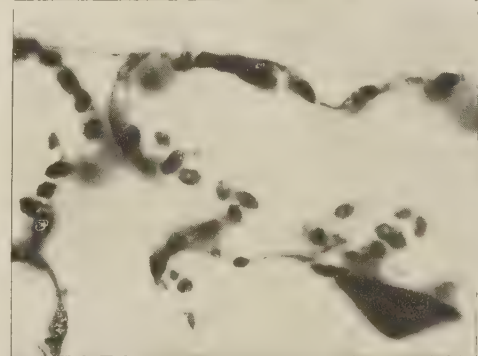


2b.

Fig. 2a, b. After plunging into liquid nitrogen, without being thawed immediately fixed with Carnoy at a) -27°C , b) $+27^{\circ}\text{C}$



3a.



3b.

Fig. 3a, b. Directly fixed with with Carnoy at a) -27°C , b) $+27^{\circ}\text{C}$. Magnif. 1500 : 1. Photomicrographs by J. Kubeč.

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Цитоморфологическое действие низких температур, изучаемых с помощью фиксаторов различной температуры

КАРЕЛ БЕНЕШ

Резюме

Исследовался механизм цитоморфологического действия низких температур на листья 4-дневных проростков пшеницы. 1. Отрезанные верхушки листьев в металлической корзинке погружались в жидкий азот и без оттаивания как можно быстрее фиксировались. 2. Таким же образом обрабатывались и следующие объекты, но после погружения в жидкий азот они оставлялись при комнатной температуре до оттаивания, и только после этого фиксировались. 3. Следующая серия объектов фиксировалась прямо, т. е. без замораживания в жидком азоте. Фиксаторы доводились до температуры —76, —27, +27 и +56° C. Фиксация в спиртово-уксусной смеси Carnoy-a 20 мин., в смеси Lison-a 2 часа. Обезвоживание и заливка производились на вибраторе. Срезы окрашивались кислым фуксином по Drawert-у и железным гематоксилином по Regaud. Результаты представлены в таблице (наблюдения хлоропластов верхушки первого зеленого листа):

	—76° C	—27° C	+27° C	+58° C
Из жидкого азота прямо в фиксатор	нормальные	нормальные	неправильные образования и нормальные	неправильные образования и нормальные
Из жидкого азота 10-мин. оттаивание и потом фиксатор	неправильные образования	неправильные образования	неправильные образования	неправильные образования
Прямо в фиксатор	деформированные и нормальные	деформированные и нормальные	нормальные	нормальные

Одинаковым способом замороженные, но различным образом оттаявшие объекты дают неодинаковые результаты. Отсюда вытекает, что разрушение хлоропластов происходит при оттаивании тканей. Необходимо однако допустить, что в случаях медленного замораживания, в опытах при высшей температуре (-20°C), или если объект слишком велик, разрушение может наступать и при замораживании. Разрушение хлоропластов наступает одновременно с разрушением ядра, и не все хлоропласты одновременно разрушаются.

The Effect of β -Indolebutyric Acid on Phosphatase and Catalase Activity in Grape Vine Cuttings

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Souhrn

Autor sledoval účinek kyseliny β -indolylmásečné (IMK) na aktivitu fosfatázy a katalázy v basálních částech řízků vinné révy odr. Müller-Turgau. Řízky se dvěma pupeny z jednoletých výhonů byly spodním koncem ponořeny na 3—5 sec do roztoku stimulatoru (500 mg IMK v 1 l 50% ethanolu) a poté napichány do písku v množárně. Aktivita obou enzymů byla sledována v období od stimulace do objevení kalusu a prvních kořínků při teplotách 15, 25, 35, 45, 55, u fosfatázy i při 65 a 75° C. Aktivita fosfatázy byla měřena rychlostí rozkladu 0,1 M Na-glycero-fosfátu, při čemž uvolněný fosfor byl stanoven podle MARTINA a DOTYHO (1949) modifikací HAUGE, KING, CHELDELINA (1955). Aktivita katalázy byla stanovena metodou podle APPELMANA (1951), modifikovanou KUBÍNEM (1956).

Při všech teplotách, při nichž byla aktivita enzymů měřena, se vliv IMK projevilo zvýšením aktivity fosfatázy a snížením aktivity katalázy. Přesto, že rozdíly mezi aktivitami tkání stimulovaných a kontrolních nejsou příliš veliké (průměrně asi 20 %), jsou ve většině případů statisticky průkazné a vyskytují se ve všech pokusech, jež byly provedeny. Při přepočtu získaných hodnot na organický dusík jsou rozdíly mezi aktivitou tkání stimulovaných a kontrolních menší u fosfatázy a větší u katalázy, než při přepočtu na sušinu. IMK urychluje tvorbu kořenů. Příčinou toho však není jen kvantitativní zrychlení, nýbrž zřejmě i hlubší kvalitativní změna metabolických procesů. Svědčí o tom paralelní průběh změn aktivit obou enzymů ve tkáni stimulované a kontrolní.

Summary

Phosphatase and catalase activity in the woody tissues of grape vine cuttings (*Vitis vinifera*), stimulated by β -indolebutyric acid, was studied at various temperatures. Its influence is evinced by increased root formation. The cause

of the final effect is not, however, only quantitative acceleration of the metabolic processes. The parallelism of the activity changes of both enzymes in the stimulated and control tissues is evidence of this.

β -indolebutyric acid significantly increases the activity of phosphatase and decreases that of catalase.

Introduction

The function of phosphatase and catalase is usually discussed in direct relation to the growth processes and to the primary effect of auxins. The close connection between their activity and growth has been established particularly in experiments in which the influence of auxins on the growth of isolated plant tissues and organs has been studied (BONNER and BANDURSKI 1952, LARSEN 1951, OVERBEEK van 1952).

In this work we have aimed at obtaining information about the changes in the activity of both enzymes in the woody tissues of grape vine cuttings which had been acted upon by β -indolebutyric acid (IBA).

Materials and Methods

Enzyme activity was ascertained in the basal parts of two bud cuttings of grape vine (*Vitis vinifera*), variety Müller-Turgau, in the period between stimulation to the appearance of callouses and the first roots. The lower ends of the cuttings, taken from one-year shoots in the usual way (the lower incision was slightly oblique, about 0.5 cm. below the bud), were plunged immediately after separation up to the bud in a solution of the stimulator for 3—5 secs. (500 mg. IBA in 1 litre of 50% pure ethanol solution). The control solution did not contain IBA. The ends of the cuttings were then pushed to the depth of 1 cm. into river sand which had been washed with water and sieved. The sand was laid on a substratum of coke and peat on a shelf in the glass house with heating from a steam pipe from below. Its humidity was maintained during the experiment, by daily spraying at about seven per cent by weight of water. The temperature of the sand was 22 to 24°C, the air temperature 17 to 20°C and the relative humidity of the air about 75%. The experiments were carried out between October and March in 1955—56 and 1956—57.

In the pilot trial of the effect of IBA on the material used, its effect on the rate of root formation and on the accumulation of nitrogen in the stimulated tissues was observed. At intervals of several days following stimulation the number of clearly perceptible roots, the dry weight of the roots and the content of organic nitrogen in the tissues above the cut surface were determined (graphs 1, 2, 3). Nitrogen was determined in pulp, prepared in the same way as for the study of enzyme activity, by the usual micro Kjeldahl method (NIEDERL and NIEDERL 1946).

The activity of phosphatase was determined by a method adapted after several authors (COURTOIS et TRAN VAN AI 1953, IGNATIEFF and WASTENEYS 1936, MARTIN and DOTY 1949). Into each of three 50 ml. Erlenmeyer flasks A, B, C, 0.2 g. of fresh pulp was placed which was obtained by grating about three mm. of wood from the bases of the cuttings, from which the bark and pith had been removed. The vessels contained the following solutions: A — 2 ml. of buffer, B — 4 ml. of buffer, C — 1 ml. of buffer, and 1 ml. of trichloroacetic acid (TCA). The flasks were then fixed to the shaker of a Warburg apparatus, being at the same time submerged in a tempered water bath. After five minutes of shaking, when temperature equilibrium had been attained, 2 ml. of tempered solution of glycerophosphate was added to A and C and the flasks were stoppered. Incubation lasted 1 hour with continual shaking at 100 oscillations per minute. The reaction was interrupted by the addition of 1 ml. of TCA to A and

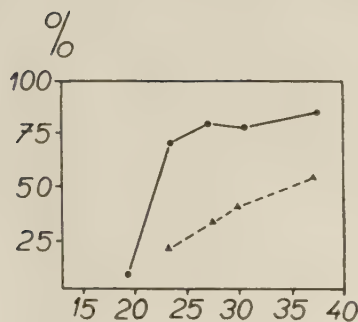


Fig. 1.

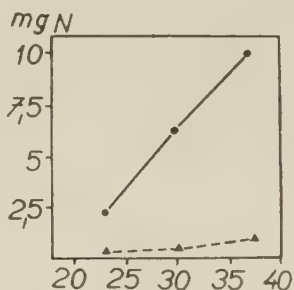


Fig. 2.

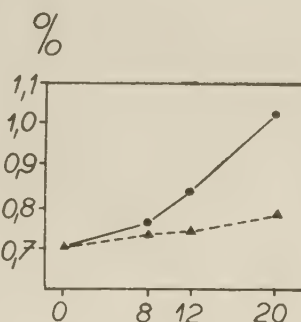


Fig. 3.

Fig. 1. Influence of IBA on rate of rooting of cuttings. Ordinate — per cent of rooted cuttings, abscissa — number of days after stimulation. Continuous line — stimulated cuttings, broken line — control. Each value corresponds to fifty cuttings.

Fig. 2. Influence of IBA on rate of production of root dry weight. Ordinate — dry weight in mg., abscissa — number of days after stimulation. Continuous line — stimulated cuttings, broken line — control. Each value corresponds to fifty cuttings.

Fig. 3. Influence of IBA on accumulation of organic nitrogen at the stimulated base of cuttings. Ordinate — per cent of organic nitrogen in dry weight, abscissa — number of days after stimulation. Continuous line — stimulated cuttings, broken line — control. Each value is the average of five measurements. For each measurement material from ten cuttings was used.

B.; 1 ml. of buffer was added to C. The reaction mixture was filtered through paper filters on which the dry weight and organic nitrogen were then determined. The following solutions were used: 0.1M Na-glycerophosphate (mixture of both isomers), 1 N acetate buffer pH 5.4 (KLEINZELLER, MÁLEK and VRBA 1954) and 20% TCA.

The regularity of the reaction and the fact that the values measured could be reproduced made it possible to avoid homogenisation of the samples and to use pulp for the determination of the activity of phosphatase.

The quantity of phosphate which had passed during incubation from the tissue into the surrounding solution was ascertained in controls without substrate. In the controls with substrate and TCA the non-enzymatic decomposition of glycerophosphate was studied. In controls with TCA and substrate and with TCA without substrate the quantity of freed phosphorus was almost identical and about one third less than in controls without substrate and TCA. From the values for the experimental samples therefore only the corresponding values of control B were subtracted. Phosphorus was determined by MARTIN and DOTY's method (1949) as modified by HAUGE, KING, CHELDELIN (1955). The intensity of blue stain with molybden blue was measured on the photoelectric colorimeter and the values measured were converted to mg. P corresponding to the unit of dry weight and nitrogen of the sample concerned. The linearity of the reaction for several hours from the beginning made it possible to use values corresponding to the quantity of phosphate freed during one hour as the means of expressing the relative activity of the enzyme.

The activity of catalase was determined by APPELMAN's method (1951) as modified by KUBÍN (1956). 2 ml. of Sørensen's phosphate buffer of pH 6.9 was pipetted into the main chamber of a Warburg vessel and 0.05 g. of fresh pulp added; into the side-arm 0.5 ml. of 1% hydrogen peroxide was pipetted. The vessel was attached to a manometer and tempered for 10 min. in a water-bath with simultaneous shaking (100 oscillations per minute). After this the level of the column of Brodie's solution was adjusted in the closed arm of the manometer to zero and in the open arm to 30. After pouring the peroxide into the main chamber of the vessel the rise of the meniscus in the open arm of the manometer was registered with the aid of the semiautomatic optic method (KUBÍN 1956). The vessel was protected against changes in temperature during withdrawal from the water bath by a rubber cover filled with water.

The course of the reaction was followed from the start for 1—2 minutes, in which time 27 or more measurements were taken. These were converted in order to correspond with the course of the reaction when the volume of the gaseous phase in the closed arm of the manometer is constant and with the amount of H_2O_2 consumed. The values obtained during the first ten seconds were not included. The sample was transferred to filter paper after the reaction had finished, in order to determine the dry weight and organic nitrogen. During the first minute the course of the reaction was practically linear at temperatures at which heat denaturation does not take place. In order to express the activity of the enzyme we therefore used values corresponding to the amount of μM H_2O_2 consumed per second.

Results

The activity of both enzymes in grape vine cuttings was studied at temperatures of 15, 25, 45, 55° C and for phosphatase in addition at 65 and 75° C. At all temperatures the effect of IBA following stimulation and planting of the cuttings in sand was evinced by increased activity of phosphatase and decreased activity of catalase. In spite of the fact that the absolute values of the differences between the activity of stimulated tissues and the controls are not particularly large (average about 20%), these differences are in the majority

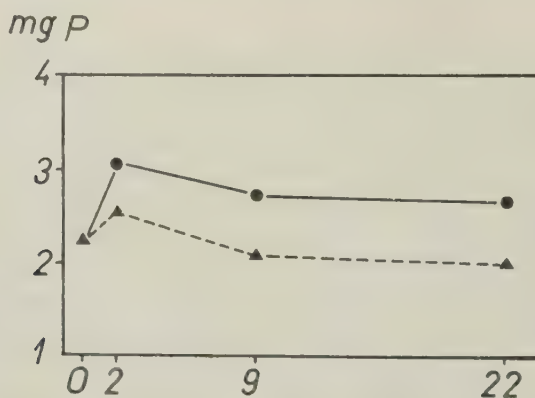


Fig. 4. Influence of IBA on activity of phosphatase. Ordinate — mg. phosphorus freed during one-hour incubation of pulp in solution Na glycerophosphate at 35° C, related to 1 mg. nitrogen. Abseissa — number of days after stimulation. Continuous line — stimulated cuttings, broken line — control. Each value is the average of five measurements. For each measurement material from five cuttings was used.

of cases statistically significant and occur in all the experiments performed. After conversion of the values obtained to organic nitrogen the differences between the activity of stimulated and control tissues are smaller for phosphatase and larger for catalase than in the case of the conversion to dry weight (Fig. 4, 5).

Discussion

On the basis of experimental findings BONNER and WILDMAN (1947) declared that native heteroauxin in conjunction with specific protein forms a so-called auxin protein which, under certain conditions, acts like phosphatase. It frees energy contained in phosphate compounds, which can be used for synthetic reactions and growth. This theory does not seem to have been confirmed, but nevertheless some work indicates that there may be a certain connection between phosphatase and growth (COURTOIS and TRAN VAN AI 1953, IGNATIEFF 1937, ROBINSON and BROWN 1954, TODD 1951).

The significance of phosphatase for cell metabolism was elucidated by the work of AXELROD (1948) and MEYERHOF and GREEN (1950). They demonstrated that phosphatases can transfer the phosphate group and function like phosphokinases. From this the importance the phosphatases or phosphokinases for growth is clearly evident. It is, for example, known (KREBS 1957) that the hexokinase reaction glucose—glucoso-6P in many cases determines the rate of respiration and of glucose consumption. Together with glyceraldehydephosphate dehydrogenase it also determines the magnitude of the production of energy from glucose and the level of acids, the aminization of which leads to the origin of proteins.

The direct relation of phosphatase, and of other enzymes of the energy metabolism, to growth is in contrast to the behaviour of catalase, the activity of which is in many cases, as has been shown in a number of experiments, in inverse ratio to growth (DELEANO, ULMANN and ULMANN 1940, DYKYJ-SAJFERTOVÁ 1956, GALSTON 1951). The relation of catalase to the enzymatic system, which oxidises heteroauxin in the plant, is explained by the fact that catalase reduces the level of hydrogen peroxide, which is simultaneously the substrate of the above enzymatic system and thus prevents its inactivation (GALSTON 1950, GOLDACRE 1951). This is in agreement with the finding that with a high content of heteroauxin in plant organs at rest there is a correspondingly high concentration of catalase. It does not, however, answer the question of why the activity of catalase is reduced by the action of auxins at concentrations which activate growth. One of the possible explanations is that auxins, which have penetrated into cells where their concentration becomes inhibitional for growth, are oxidised by peroxidase which at the same time

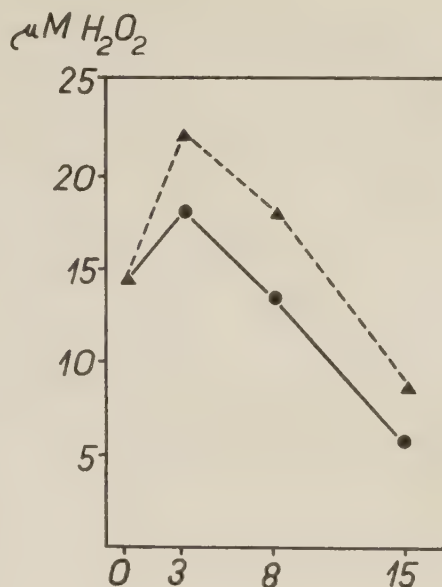


Fig. 5. Influence of IBA on activity of catalase. Ordinate — $\mu\text{M H}_2\text{O}_2$ decomposition of pulp in 1 sec. at 35°C , related to 10 mg. nitrogen. Abscissa — number of days after stimulation. Continuous line — stimulated cuttings, broken line — control. Each value is average of five measurements. For each measurement material from four cuttings was used.

represses catalase activity. On the other hand it is known that catalase itself can function like peroxidase (KEILIN and HARTREE 1945) and even oxidise indolelactic acid (WEYGOOD and MACLACHLAN 1950). The relation between the reduction of catalase activity and growth is evidently of more general significance, for low activity was ascertained not only in plant tumours (GALSTON 1951), but also in cases of malignant growth in animal tissues (BROWN and WALTERS 1957).

From the results of our work it is clear that the changes in the stimulated tissues are twofold. On the one hand there are those which are in agreement with changes in the control: rise in activity, maximum and decrease. On the other hand the absolute increase in phosphatase activity and reduction in catalase activity. The influence of IBA is evinced in more rapid root formation. The fact that the actual cause of the final effect is, however, not only the quantitative acceleration of metabolic processes, can be seen from the parallel course of enzyme activity in stimulated and control tissues. If only an acceleration were involved the control curves would be shifted to the right.

The most recent work of some authors studying the action of auxins, for example BUSSE and KANDLER (1956), indicates that the effect of auxins on growth in length of coleoptiles is primarily in the induction of growth processes related to the cell walls, while changes in the intensity of processes of the energy supply of the organism occur secondarily. During the embryonal growth of cells, such as is involved in our case, the effect of auxins is, however, evidently of more fundamental nature. If IBA were to act primarily on the cell walls of the latent meristem the increase in size of the cells would be prevented by the pressure of the surrounding woody tissues. Changes in the activity of a phosphatase and catalase appeared, however, much sooner than the growth of cells, accompanied by a transfer of organic nitrogen to the stimulated tissues. This was also confirmed by microscopic investigation.

I wish to thank Prof. Dr. S. Prát for his interest and help. I am indebted to J. Opřt for help in connection with the glass house experiments.

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Действие β -индолилмасляной кислоты на активность фосфатазы и каталазы в черенках виноградной лозы

ИРЖИ ЛУШТИНЕЦ

Резюме

Автор исследовал действие β -индолилмасляной кислоты (ИМК) на активность фосфатазы и каталазы в базальных частях черенков виноградной лозы разновидности *Müller-Turgau*. Черенки с двумя глазками из однолетних побегов погружались на 3—5 секунд нижним концом в раствор стимулятора (500 мг ИМК в 1 л 50 % этилового спирта), после чего помещались в песок для укоренения. Активность обоих энзимов исследовалось в период между стимуляцией и появлением калюса и первых корешков при температурах в 15, 25, 35, 45, 55, а у фосфатазы — и при 65 и 75° С. Активность фосфатазы измерялась скоростью разложения 0,1 М глицерофосфата натрия, причем освобождающийся фосфор определялся по Martin-у и Doty (1949) — модификации Nauge, King, Cheldelin-a (1955). Активность каталазы определялась по методу Appel-man-a (1951) в модификации Kubín-a (1956).

При всех температурах, при которых активность энзимов измерялась, влияние ИМК проявлялось в повышении активности фосфатазы и в понижении активности каталазы. Несмотря на то, что разница между активностями стимулированных и контрольных тканей не слишком велика (в среднем около 20 %), в большинстве случаев она оказывается статистически значимой: она наблюдалась во всех поставленных нами опытах. При пересчете полученных величин на органический азот разница между активностью стимулированных и активностью контрольных тканей у фосфатазы бывает меньше, а у каталазы больше, чем при пересчете на сухое вещество. ИМК ускоряет образование корней. Однако причиной этого является не только количественное ускорение, но, по-видимому, и более глубокое качественное изменение процессов обмена веществ, как о том свидетельствует параллелизм изменений активности обоих энзимов в стимулированной и контрольной тканях.

Über den Einfluss einiger Humusstoffgruppen auf den Gehalt der oberirdischen Weizenteile an reduzierenden und nicht reduzierenden Zuckern

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Souhrn

Vliv alkoholbenzenové frakce, frakce huminových kyselin a frakce fulvokyselin působících v koncentraci 0,1; 1; 10 a 100 mg/l se projevuje na rovnováze glycidů v nadzemní části mladých rostlin pšenice takto: Alkoholbenzenová frakce způsobuje pokles obsahu cukrů neredukujících (N) a zvýšení obsahu cukrů redukujících (R), takže poměr N : R je snižován. To lze pokládat za příznak zvýšení intenzity katabolických pochodů. Frakce fulvokyselin tento poměr zvyšuje, z čehož je možno usuzovat na větší intenzitu pochodů skladebných. Působení frakce huminových kyselin je málo výrazné. Pozorované změny souvisí se změnami celkové váhy sušiny tak, že vysoký poměr N : R vyvolaný frakcí fulvokyselin je spojen s přírůstkem celkové váhy sušiny, jeho nízká hodnota způsobená alkoholbenzenovou frakcí nikoliv.

Zusammenfassung

Der Einfluss der Alkohol-Benzen-Fraktion, der Fraktion der Huminsäuren sowie der Fraktion der Fulvosäuren, die in Konzentrationen von 0,1; 1; 10 und 100 mg je Liter wirkten, äussert sich im Gleichgewichte der Glycide in den oberirdischen Teilen junger Weizenpflanzen folgendermassen: Die Alkohol-Benzen-Fraktion bewirkt ein Absinken des Gehaltes an nicht reduzierenden Zuckern (N) und ein Ansteigen des Gehaltes der reduzierenden Zucker (R), sodass der Quotient N : R herabgesetzt wird. Dies lässt sich als Anzeichen einer Erhöhung der Intensität katabolischer Vorgänge erklären. Die Fulvosäurenfraktion dagegen erhöht dieses Verhältnis, sodass sich auf eine Erhöhung

der Intensität der Synthese schliessen lässt. Die Wirksamkeit der Huminsäurenfraktion ist wenig ausdrucksvoll. Die Beobachtungen dieser Veränderungen korrespondieren mit den Änderungen des gesamten Trockengewichtes auf solche Weise, dass ein von der Fulvosäurenfraktion bewirkter hoher Quotient $N : R$ mit einem Zuwachs des gesamten Trockengewichtes verbunden ist, während es sein durch die Alkohol-Benzen-Fraktion bewirkter niedriger Wert keineswegs ist.

Einführung

In der vorhergehenden Mitteilung berichtete ich über den Einfluss der Alkohol-Benzen-Fraktion des Bodenhumus, der Huminsäuren- und der Fulvosäurenfraktion auf die Gewichtszunahme junger Pflänzchen des Frühjahrsweizens (TICHÝ 1958). Wachstums- und Wassergehaltsänderungen, die Verteilung der Trockensubstanz in den Pflanzenorganen und Änderungen ihres Bildungsvorganges, hervorgerufen durch die genannten Stoffe, sind von den übrigen metabolischen Prozessen der Pflanze nicht zu trennen und darum hat eine ganze Reihe von Autoren (Siehe Literaturverzeichnis PRÁT 1955) ihr Augenmerk auf das Studium des Einflusses von Humusstoffen auf die Mineralabsorption, die Atmung und die Stickstoffbindung gelenkt. Neuerdings beachten PRÁT (1955), PRÁT, ČATSKÝ und MELICHAR (1957) sowie ČATSKÝ (1958) diese Einflüsse und weitere Veränderungen im Metabolismus des Stickstoffes. Änderungen im Kohlenhydratstoffwechsel, der sehr eng mit dem Wachstum verbunden ist, wurden bis jetzt allgemein ausser acht gelassen. Vor kurzem berichteten FLAIG, SCHARER und SCHOLL (1957) über den Einfluss des Thymohydrochinons als Humusstoffmodells auf den Kohlenhydratstoffwechsel des Getreides. Sie stellten fest, dass dieser Stoff den Gehalt an reduzierenden Zuckern erhöht und umgekehrt den Stärkegehalt herabsetzt. Seine Wirkung erklären sie durch den hemmenden Einfluss auf die Stärkesynthese und die Wirkung mancher Fermente des Cyklus der Tricarbonsäuren, bzw. die Förderung der Aktivität hydrolytischer Fermente.

Im Hinblick auf die Tatsache, dass der Pflanzenwuchs in engem Zusammenhange mit dem Kohlenhydratstoffwechsel steht, erachte ich es für günstig, meine vorhergehende Arbeit durch diesen Bericht über den Einfluss der Alkohol-Benzen-Fraktion, der Huminsäurenfraktion und der Fulvosäurenfraktion auf den Gehalt an reduzierenden und nicht reduzierenden Zuckern in den oberirdischen Teilen 10 Tage alten Weizens zu ergänzen.

Material und Methode

Für die Versuche habe ich Frühjahrsweizen „Niva“ benutzt, den ich in Blumentöpfe mit Sand ausgesät habe, welcher vorher einmalig jeweils mit einer der Humusfraktionen angesogen war (Alkohol-Benzen-Fraktion, Huminsäurenfraktion und Fulvosäurenfraktion), deren Konzentration 0 (Kontrolle); 0,1; 1; 10 und 100 mg per Liter betrug. Die Herstellung dieser Fraktionen ist in der bereits erwähnten Arbeit beschrieben (TICHÝ 1958). In diesem Milieu wurde

der Weizen 10 Tage lang gezüchtet, dann wurden die oberirdischen Teile abgeschnitten, rasch getrocknet und auf den Gehalt an reduzierenden und nicht reduzierenden Zuckern mittels der Methode nach Bertrand geprüft (JERMAKOV et al., 1952).

Neben diesen Sandkulturen, die jeweils Alkohol-Benzen-Fraktion, Huminsäurenfraktion oder Fulvosäurenfraktion enthielten, habe ich eine weitere Versuchsserie angelegt, bei denen der Sand einmalig mit einer Mischung von Lösungen der Huminsäurenfraktion (HK) und der Fulvosäurenfraktion (FK) in einer Gesamtkonzentration von 2,5 mg je Liter angesogen war. Die einzelnen Varianten unterschieden sich nur durch das Verhältnis dieser beiden Fraktionen. Es sind folgende Mischungen hergestellt worden: 10 : 0 (nur HK), 8 : 2, 6 : 4, 4 : 6, 2 : 8 und 0 : 10 (nur FK).

In dieser Versuchsserie, ähnlich wie bei den Fulvosäuren in der vorhergehenden Serie, wurde bei den einzelnen Varianten der Kaliumsulfatgehalt durch Zugabe der errechneten Menge angeglichen. (Siehe auch TICHÝ 1958.)

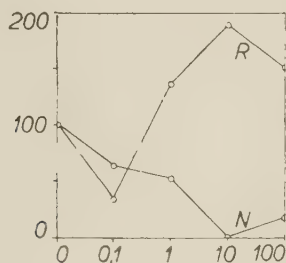


Abb. 1.

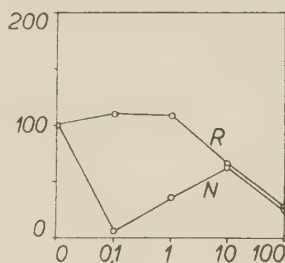


Abb. 2.

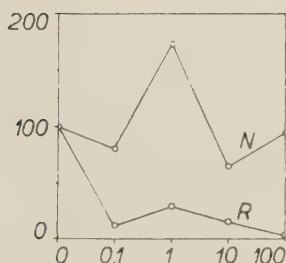


Abb. 3.

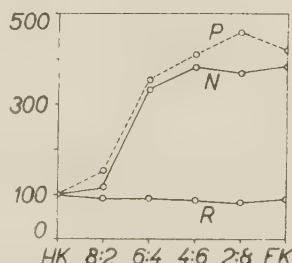


Abb. 4.

Abb. 1—3. Einfluss steigender Konzentration der Alkohol-Benzen-Fraktion (Abb. 1), der Huminsäuren-Fraktion (Abb. 2) und der Fulvosäuren-Fraktion (Abb. 3) auf den Inhalt der reduzierenden (Kurve R) und nicht reduzierenden Zucker (Kurve N) in oberirdischen Weizenteilen.

Achse x: Konzentration der Fraktionen in mg/L

Achse y: Zuckergehalt ausgedrückt in % der Kontrolle.

Abb. 4. Einfluss der verschiedenen Verhältnisse der Fraktion der Huminsäuren (HK) und der Fraktion der Fulvosäuren (FK) in einer Mischung von einer Gesamtkonzentration von 2,5 mg/L auf den Gehalt der reduzierenden (Kurve R) und nicht reduzierenden Zucker (Kurve N) in oberirdischen Weizenteilen. Die Kurve P veranschaulicht die Änderungen des Verhältnisses N : R.

Achse x: Verhältnis HK : FK in der Mischung.

Achse y: Inhalt der Zucker, bzw. Quotient N : R ausgedrückt in Prozenten der Kontrolle (bezogen auf die Huminsäuren-Fraktion).

Ergebnisse

Die Ergebnisse der Analysen sind in den Diagrammen 1 bis 4 graphisch dargestellt. Sie erweisen, dass Humusstoffe auf das Zuckergleichgewicht in der Pflanze einen besonderen Einfluss haben und dass dieser Einfluss sich mit der Konzentration der genannten Stoffe im Substrate ändert.

Wenn die Pflanze im Sande mit Alkohol-Benzen-Fraktions-Beigabe gezüchtet worden ist, kam es bei der Steigerung der Konzentration dieser Stoffe zu einer Herabsetzung des Gehaltes an nicht reduzierenden Zuckern und umgekehrt zu einer Erhöhung des Gehaltes der reduzierenden Zucker (mit Ausnahme der niedrigsten Konzentration — Abb. 1). Bei Änderungen des Inhaltes dieser Zucker-Fractionen ändert sich auch ihr gegenseitiges Verhältnis. Durch den Einfluss der Alkohol-Benzen-Fraktion erscheint der Zahlenwert des Verhältnisses der nicht reduzierenden zu den reduzierenden Zuckern mit Ausnahme des Wertes bei der allerniedrigsten Konzentration allgemein herabgesetzt. Beim Weizen, welcher auf Sand mit Huminsäurenbeigabe wuchs, sank der Gehalt an reduzierenden Zuckern und zwar bis zur Verwendung der höchsten Konzentration dieser Fraktion (Abb. 2). Der Gehalt an nicht reduzierenden Zuckern sinkt zwar anfangs gleichfalls, später vergrößert er sich jedoch und erreicht bei der Konzentration von 10 mg je Liter ein Maximum, worauf er wieder sinkt. Infolge dieser Änderungen erscheint das Verhältnis der Menge der nicht reduzierenden Zucker zur Menge der reduzierenden nur bei den schwächeren Konzentrationen herabgesetzt, während es bei einer Konzentration von 10 und 100 mg je Liter wieder die Werte der Kontrolle erreicht. Durch die Wirkung der Fulvosäuren kommt es zu einer noch stärkeren Erhöhung des Verhältnisses der Menge der nicht reduzierenden Zucker zur Menge der reduzierenden (Abb. 3). Das erweisen auch die Ergebnisse eines Vegetationsversuches auf Mischungen der Huminsäuren-Fraktion mit der Fulvosäuren-Fraktion, bei welchen die maximalen Verhältniswerte der Menge der nicht reduzierenden Zucker zur Menge der reduzierenden Zucker gerade auf der Seite der Fulvosäuren erreicht werden (Abb. 4).

Diskussion

Wie im experimentellen Teile gezeigt worden ist, greifen die Humusstoffe auch in die metabolischen Vorgänge ein, wie es die Bildung und das Gleichgewicht einiger Zucker sind. Dieses Ergebnis hängt sehr eng mit den Ergebnissen der vorhergehenden Arbeit über den Einfluss der Humusstoffe auf das Wachstum des Weizens zusammen (Tichý 1958), vertieft sie und klärt einige Zusammenhänge der studierten Erscheinungen.

Lvov (1952) macht darauf aufmerksam, welch bedeutsamer Indikator der Vitalität der Pflanzen das Verhältnis der Saccharose zu den Monosen ist, bzw. das Verhältnis der nicht reduzierenden Zucker zu den reduzierenden. Ein hoher Wert dieses Quotienten ist ein Kennzeichen dafür, dass synthetische Vorgänge den Katabolismus der Pflanze überwiegen und dadurch ein Kenn-

zeichen ihrer hohen Vitalität. Ein niedriger Wert des Verhältnisses der nicht reduzierenden Zucker zu den reduzierenden ist hinwiederum ein Kennzeichen dafür, dass aufspaltende Vorgänge überwiegen, die zu einer Verlustbilanz des pflanzlichen Organismus führen.

Die Alkohol-Benzen-Fraktion ist darum von diesem Gesichtspunkte aus kein Faktor, den man als günstig bezeichnen kann. Sie ruft ein Absinken des Verhältnisses der nicht reduzierenden zu den reduzierenden Zuckern hervor, was auf überwiegende Aufspaltungsvorgänge hinweist. Das stimmt sehr gut mit der Feststellung überein, dass durch die Einwirkung dieser Fraktion keine Erhöhung des Trockengewichtes der ganzen Weizenpflänzchen gleichen Alters erzielt werden konnte (TICHÝ 1958). Die Fraktion der Huminsäuren setzt das Verhältnis der nicht reduzierenden Zucker zu den reduzierenden nur bei niedrigeren Konzentrationen herab. Damit hängt wahrscheinlich auch die Tatsache zusammen, dass diese Konzentrationen auch das Trockengewicht nicht erhöhen, wie sich gleichfalls aus der angeführten Arbeit ergibt. Das hohe Verhältnis der nicht reduzierenden Zucker zu den reduzierenden bei den Fulvosäuren zeugt vom Überwiegen der synthetischen Tätigkeit der Pflanze und ist hier eng verbunden mit einer Erhöhung des Gesamt-Trockengewichtes bis zu optimalen Konzentrationen. Der verschiedene Wert der Huminsäuren-Fraktion und der Fulvosäuren-Fraktion ist schliesslich auch gut aus dem Versuche mit den Mischungen dieser beiden Fraktionen ersichtlich: Während der Inhalt der reduzierenden Zucker von der Anwesenheit beider Faktoren so gut wie unabhängig ist, steigt der Inhalt der nicht reduzierenden Zucker (und mit ihm auch ihr Verhältnis zu den reduzierenden) mit dem Steigen des Anteiles an Fulvosäuren-Fraktion (Abb. 4).

Allgemein lässt sich sagen, dass die Humusstoffe auf das Kohlenhydratgleichgewicht in den oberirdischen Teilen junger Weizenpflanzen einen besonderen Einfluss haben. Die Alkohol-Benzen-Fraktion setzt den Wert des Verhältnisses der nicht reduzierenden Zucker zu den reduzierenden herab, worin auch ihre Förderung aufspaltender, katabolischer Prozesse begründet ist. Die Fulvosäurenfraktion erhöht dieses Verhältnis, was hinwiederum von einer Unterstützung anabolischer Vorgänge zeugt. Die Wirkung der Huminsäurenfraktion ist weniger ausdrucksvoll.

Die Ergebnisse FLAIGS, SCHARRERS und SCHOLLS (1957) zeigen, dass Thymohydrochinon nur teilweise als eine Humusstoff-Modellsubstanz angesehen werden kann, denn im Charakter ihrer Wirksamkeit auf den Kohlenhydrat-Inhalt ähnelt sie am ehesten der Alkohol-Benzen-Fraktion des natürlichen Humus. Die Erhöhung des Glukosegehaltes und die Herabsetzung des Stärkegehaltes spricht nämlich gleichfalls von einer Unterstützung der Aufspaltungsprozesse, bzw. von einer Inhibition der Synthesevorgänge. FLAIG, SCHARER und SCHOLL erläutern diesen Effekt als Hemmung der Stärkesynthese, als Inhibition einiger Fermente des Cyklus der Tricarbonsäuren und als Unterstützung hydrolytischer Fermente. Auf Grund eigener und zitierter Ergebnisse, wie auch auf Grund dessen, was von der Synthese und Spaltung von Saccharose und Stärke bekannt ist, darf man vermuten, dass auch das Phosphorylasensystem von der Wirkung der Humusstoffe betroffen wird. Die Klärung dieser Frage hängt jedoch von einer weiteren experimentellen Bearbeitung ab.

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О влиянии некоторых групп гумусовых веществ на содержание редуцирующих и передуцирующих сахаров в надземной части пшеницы

ВЛАДИМИР ТИХЫ

Резюме

Влияние алкогольбензеновой фракции, фракции гуминовых кислот и фракции фульвокислот, действующих в концентрациях 0,1; 1; 10 и 100 мг/л сказывается на равновесии глицидов в надземных частях молодых растений пшеницы следующим образом: алкогольбензеновая фракция вызывает падение содержания передуцирующих сахаров (N) и повышение содержания редуцирующих (R), следовательно, отношение N : R снижается. Это возможно считать признаком повышения интенсивности катаболических процессов. Фракция фульвокислот, наоборот, повышает это отношение; следовательно, возможно судить о большей интенсивности строительных процессов. Действие фракции гуминовых кислот менее резко выражено. Наблюдаемые изменения связаны с изменением веса сухого вещества таким образом, что высокое отношение N : R, вызываемое фракцией фульвокислот, связано с ростом веса сухого вещества; не так его низкое отношение, вызываемое алкогольбензеновой фракцией.

A New Strain of Tobacco Mosaic Virus Caused by Influencing the Metabolism of the Host

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Souhrn

V práci je popsán nový kmen viru tabákové mosaiky, který podle svých charakteristických páskových příznaků („Stripe“, „Streifen“) byl nazván S-kmenem VTM. Jako výchozího materiálu bylo použito Al-kmene viru tabákové mosaiky.

Na rozdíl od metod až dosud používaných byl S-kmen získán přímým zásahem do metabolismu hostitelské rostliny, přivoděným změnou jejího životního prostředí. (SVOBODOVÁ 1954, 1958). Bylo použito obvyklých hostitelů viru tabákové mosaiky, především rostlin tabáku a rajčat. Tyto rostliny byly vypěstovány za aseptických podmínek na agarových půdách v Erlenmeyerových baňkách při snížení dávek živin, za zkráceného světelného dne a při omezené výměně vzduchu, takže zakrslly. Po pasáži výchozího kmene VTM (Al-kmene) těmito zakrslými rostlinami došlo ke změnám příznaků, které se projevíly teprve v dalších pasážích na rostlinách vypěstovaných za normálních podmínek ve skleníku v zahradní půdě, kde měl virus zase normální možnost se množit. Z dosažených příznaků byla dále sledována jen změna nejvýraznější (páskové příznaky S-kmene). Tímto střídavým pasážováním Al-kmene VTM rostlinami normálními a zakrslými, pěstovanými v umělém živném prostředí, tj. dvoj- nebo i trojnásobnou náhlou změnou životního prostředí viru, byl získán nový kmen (S-kmen) celkem pětikrát v různých ročních dobách. Po stránce symptomatické nebyly zjištěny mezi jednotlivými reprodukci této změny rozdíly. Výsledky byly také stejné, pocházeli výchozí materiál z rostlin rajčat nebo z tabáků odrůdy Samsun.

Na vznik nového kmene nepůsobila jen zakrslost hostitelských rostlin, jak ukázaly pasáže viru zakrslými rostlinami na mikrozáhonu ve volném prostředí, ale bylo třeba ovlivnění metabolismu abnormálním prostředím v Erlenmeyerově baňce ještě po očkování.

Po zpětných pasážích S-kmene z rostlin, vypěstovaných běžným způsobem ve skleníku, na rostliny se zakrslým růstem v umělém živném prostředí k dalším změnám nedošlo. Také pasáže na deseti jiných hostitelích pěstovaných běžným způsobem v zahradní půdě i v umělém živném prostředí žádnou další změnu příznaku nepřivodily. S-kmen je udržován stálými pasážemi na *Nicotiana tabacum* Samsun po dobu šesti let, přičemž každoročně dochází k hromadnému očkování na velký počet rostlin. Považujeme tedy dosaženou změnu za trvalou.

Ke vzniku nového kmene postačilo nepřímé ovlivnění viru prostřednictvím pozměněného metabolismu hostitelské rostliny. Změna se projevila až po pasáži normálními, nijak neovlivněnými rostlinami, kdy reprodukce viru nebyla už omezena zpomaleným růstem hostitelské rostliny. Je určitá analogie ve vzniku S-kmene a TM 44, získaného PFANCHUCHEM a spol. (1940). TM 44 vznikl působením X-paprsků; jeho výchozím materiálem byl však obyčejný zelený kmen tabákové mosaiky. Není řešena otázka, zda S-kmen vznikl selekcí nebo mutací. Mutaci však považujeme za pravděpodobnější.

Zjištění biologických, chemických a dalších vlastností S-kmene a jejich srovnání s vlastnostmi jeho kmene výchozího a obyčejného zeleného kmene VTM bude publikováno.

Summary

The paper describes a new strain of tobacco mosaic virus which has been named S-strain of TMV because of its characteristic stripe symptoms. The Al-strain of tobacco mosaic virus was used as the initial material.

In contrast to methods used hitherto the S-strain was obtained by interfering with the metabolism of the host plant by a change in the environment. (SVOBODOVÁ 1954, 1958). The usual hosts of tobacco mosaic virus were used, chiefly tobacco and tomato plants. These plants were cultivated under aseptic conditions on an agar medium in Erlenmeyer flasks with a reduced amount of nutrients, a shortened period of illumination and a limited air supply, so that they were stunted. Following the passage of the initial Al-strain of TMV through these dwarfed plants changes in symptoms appeared, which became apparent only after further passages through plants grown under normal conditions in a glass house in garden soil, where the virus once again had normal conditions for reproduction. Of the new symptoms obtained, only the most striking were further studied (the symptoms of S-strain). By means of alternating of the passage of the Al-strain of TMV from normal—to dwarfed—to normal plants grown under artificial nutrient conditions, i. e. a twice or thrice repeated sudden change of environment, the new strain (S-strain) was obtained five times in all and at different times of the year. From the symptomatic point of view no differences were found in the different repetitions of this change. The results were also the same for material from tomato plants and from tobacco of the Samsun variety.

The origin of the new strain was not caused only by the stuntedness of the host plants. It was also necessary to influence its metabolism by an abnormal environment (particularly insufficient air) in the Erlenmeyer flask before and after inoculation.

Following the reverse passage of the S-strain from plants grown normally in a glass house to dwarfed plants in an artificial environment no further changes occurred. Neither did the passage to ten other hosts grown normally on garden soil and in artificial nutrient produce any further change in symptoms. The S-strain has been maintained by a succession of continuous passages through *Nicotiana tabacum* Samsun for six years, and each year mass inoculation of a large number of plants is carried out. We therefore now consider the change to be permanent.

Indirect influencing of the virus through a change in the metabolism of the host plant sufficed for the induction of the new strain. Its symptoms appeared only after passage through normal unaffected plants, where the virus reproduction was not inhibited by the retarded growth of the host plant. There is a certain similarity in the emergence of the S-strain and the TM44-strain, obtained by PFANCHUCH *et al.*, with X-ray irradiation. However, the initial material was the ordinary green strain of TMV.

The biological, chemical and other characteristics ascertained for S-strain and a comparison of these with the characteristics of the initial Al-strain and the ordinary green strain of TMV will be published later.

Introduction

For the development of the large number of new strains obtained in the laboratory the effect of raised temperatures on the infected plant or insect vector (KUNKEL 1937), passage through the host plant on which the virus usually parasitises and the effect of radiation were all used. LARSON *et al.* (1955) achieved mutation by the action of nitrogen mustard. SUKHOV (1955) describes new strains which developed following infection of the host plants by various strains and species of virus (SUKHOV and KAPITSA 1956). The greatest number of strains was isolated by mechanical separation from a mixture of strains, by selection of inoculum from different parts of the leaves and from different spots. There has been no success in obtaining new strains by influencing the virus itself outside the host cell, although various chemical reagents and other agents were tried. Only the re-combination of nucleic acids and proteins of some strains of tobacco mosaic virus as carried out by FRAENKEL-CONRAT and SINGER (1957) gave rise to something like 'hybrids'.

Even though it has not been possible to obtain new strains by influencing the virus *in vitro*, some authors assume (e.g. KAPITSA 1954) that changes in the properties of the virus occur not only because of the changed metabolism of the host, but also directly because of the affecting of the biochemical reactions connected with the reproduction of the virus (e. g. raised temperatures). KAUSCHE *et al.* (1940) maintain, on the basis of their results with irradiation of plants by X-rays, that the virus is influenced secondarily through the effect on the metabolism of the host and further that this takes place only if its growth is not halted.

The aim of our work has been to obtain a strain with new properties only by direct action on the metabolism of the plant which is the usual host for the virus concerned (SVOBODOVÁ 1954).

Material and Methods

An effective way of influencing the metabolism of the host was shown to be the cultivation of dwarf plants, grown in an artificial nutrient environment and with a permanently limited air supply. The plants were grown aseptically on an agar medium in Erlenmeyer flasks with a reduc-

ed supply of nutrient, and in the summer months the period of illumination was also restricted. These dwarf plants, the metabolism of which could be regarded as abnormal, were infected with the virus so that, although plants of the same variety were used, the virus, by being passed through normal plants and then dwarfs and vice versa, was introduced into plants with a different metabolism. The first experimental plants were *Solanum lycopersicum* var. Imun, but later *Nicotiana tabacum* Samsun was found to be more suitable. At the start dwarf plants, which had reached a height of about 1 cm after five months and formed up to 10 small leaves, were used (SVOBODOVÁ 1954). Since in the course of the experiments it was found that similar results could be obtained with less stunted plants, plants were then infected which after 3—4 months of growth had attained a height of about 2—3 cm. (fig. 2), their nutrient supply being reduced to only two thirds. Tobacco plants grown normally on garden soil in the glass house were infected in about the 6th to 8th week of growth.

As the initial material for infection we used the Al-strain of tobacco mosaic virus, because of all the strains available it is symptomatically the most distinct. This strain developed in nature and was isolated by BLATTNÝ in 1953 (BLATTNÝ 1955). JAKUBOVIČ et al. (1955) determined its spectrum in ultra-violet light, its electrophoretic mobility, its qualitative and quantitative nucleoprotein aminoacid content and its morphological structure. Isolation from lesions on *Nicotiana glutinosa*, repeated many times by BLATTNÝ, using very diluted sap, never led to the separation of different forms. Nor did this take place during the mass inoculations which have been carried out each year for other purposes as well by various members of our staff since 1953, at different times of the year and under differing temperature conditions on many thousands of tobacco plants and on other species. According to JAKUBOVIČ, the solution of nucleoprotein of this virus travel in the electric field as completely homogeneous particles.

On the leaves of *Physalis Franchetti* and *P. alkekengi*, from which it was isolated, the Al-strain gives rise to dark green, whitish, yellow to yellowish-green spots, blisters and deformations. It produces the same symptoms on *Nicotiana tabacum* Samsun (fig. 1) and on *Solanum lycopersicum*. Young plants of tobacco and *Capsicum annuum* in normal environments are to a high degree killed by it and the growth of older plants is inhibited.

The process of passing and results

Work was started in the spring of 1953. Dwarf tomato plants (in groups of ten), which had been grown under aseptic conditions on an agar medium in Erlenmeyer flasks, were inoculated with sap from tomato plants systemically infected with Al-strain virus. The symptoms were very weak. The second passage was carried out on tomato plants which had been grown in the usual way on garden soil in the glass house, the third on tomatoes and on tobacco of the Samsun variety, also grown on garden soil in the glass house. The tomato plants showed atypical symptoms which were indistinct, tobacco reacted more sensitively. Two out of five tobacco plants exhibited signs of disease by a quite abnormal colouring of the tissues, never found before with the Al-strain. There were light "stripes" with a darker band within them, which followed veins of the Ist. IInd and partially IIId order (SVOBODOVÁ 1954). Further work was then directed to the following of these symptoms, since they were markedly distinct from all those previously observed. The other symptoms were not followed further and the changes obtained after the passage through plants grown on micro-beds (SVOBODOVÁ 1954) were also ignored. During the fourth passage, which was carried out again on plants grown on garden soil in the glass house, the Samsun plants showed very mild signs of disease; of five plants two were not diseased at all. In the case of three plants bulging of the intercostal areas was observed (inoculation took place in Sep-

tember 1953). This inoculation was therefore repeated on other plant species. The symptom of stripes along the veins was reproduced in three out of five plants of *Nicotiana tabacum* Virginian Gold. The other two remained without symptoms.

During the autumn and winter of 1953—1954 work was interrupted and the tobacco plants wintered in the glass house. The yellow stripes disappeared, the leaves on which they had occurred died and the blades of further leaves showed yellow and green spots (SVOBODOVÁ 1954). Further passages were carried out in the spring of 1954. Plants grown under normal conditions in the glass house were infected with sap from the spotted leaves. The stripe symptoms along the veins were reproduced regularly always in a certain proportion, the other infected plants remaining without symptoms. The general picture of the symptoms was as follows:

On evenly green leaves yellow stripes appear as the first symptom along the veins of the I—III order, on further leaves green stripes follow and patterns of light lines and stripes, to which the original stripe gives place. With higher summer temperatures and stronger sunshine, occur later symptoms of rather yellow and green patches, similar to those with the Al-strain, Ring figuring is less frequent; the extent to which patterns occur around the spots on older leaves such as are shown in fig. 6 depends on the temperature and illumination. Green stripes follow in the ontogenetical development of the plant after the yellow stripes. In milder cases of the disease, where the incubation period is longer, the yellow stripes may, however, be altogether lacking; in this case 1—2 leaves above the inoculated leaf remain without symptoms and on further leaves there appear green stripes and then spots and patterns.

The development of new symptoms was reproducible. Since it was found that the tomato is not very suitable as material, attention was again directed to the effect of other host plants. In October 1953 tobacco and tomato plants were simultaneously infected. The plants were grown aseptically on agar. The original material used in this case, as distinct from the first experiment, was sap from *Nicotiana tabacum* Samsun (fig. 1) systemically diseased with the Al-strain. In the first series it was always passed through *Nicotiana tabacum* Samsun plants on an agar medium. The plants showed indistinct symptoms until in further infections (from the 3rd to 4th) they showed none at all even when transplanted into garden soil in Petri dishes 4 cm. in diameter, where nutrients were restricted but they were in a free environment. Only after repeated infection of plants in Erlenmeyer flasks by sap from plants systemically diseased with Al-strain and grown under normal conditions in the glass house, the symptom of yellow stripes along the veins again occurred in further passages. This occurred in the following cases:

a) Following inoculation to plants in garden soil in the glass house immediately at the next passage in June 1954, but to a rather weaker degree. Clear symptoms were found only in one out of five Samsun plants, where they appeared later and as green stripes.

b) Following inoculation to plants grown on agar medium, where symptoms appeared only after the second further passage in November of the same year.

Two of the *Nicotiana tabacum* Samsun plants on an agar medium, which have been mentioned above as showing no symptoms after the third passage were transplanted into garden soil in flower pots and left in the laboratory next to plants on an agar medium. After transplantation spots appeared on these plants, in contrast to those transplanted into Petri dishes; in the further passage (the fourth), however, yellow stripes appeared, both on plants in garden soil in the glass house, where they developed in May 1954, and on plants on agar, where they developed in April of the same year.

In further passages the stripes along the veins were reproduced without change, both in winter and summer (in artificial culture on agar). Sap from plants, which exhibited stripe symptoms in August 1953, in April 1954, in June and November 1954 was used in separate passages up to twenty times and the symptoms were maintained without change. Nor was there any difference between plants of different experimental series. The results were also the same whether the original material was taken from tomatoes or tobacco var. Samsun.

The general picture of the symptoms remained unchanged whether the inoculation material was taken from individual leaves or different parts of them with yellow or green stripes, with spots and light green patterns. Also when the passages were repeated several times from Samsun plants grown in normal glass house environment to Samsun plants on agar, sap being selected for the purpose from tissues with different symptoms, the same symptom picture developed; in the first passage they were rather weak and often were seen for the first time at the green stripe stage; however, in further passages through tobacco plants on agar the yellow stripes regularly appeared as the first symptom. Following passage of the sap from normal plants back to dwarf plants in artificial nutrient there were also no further changes, only in the first passage a certain weakening of the symptoms was observed. The symptoms described became permanent on tobacco.

In order to confirm the fixation of these symptoms, sap from *Nicotiana tabacum* Samsun plants evincing these symptoms was passed to the following plants: *Nicotiana sylvestris*, *Nicotiana glutinosa*, *N. affinis*, *N. tabacum* White Burley, *N. t.* Virginia Golden, *Solanum lycopersicum*, *S. tuberosum*, *Physalis alkekengi*, *P. Franchetti*, *Capsicum annuum*.

Although individual host plants reacted differently, after passing back to *Nicotiana tabacum* Samsun the original symptom, a stripe along the veins, appeared again without change. This, together with the fact that these symptoms have been maintained without change for six years through annual mass inoculation, permits the conclusion that the change is permanent and has developed independently of the species of host and is not changed by passage through the host plants named.

During the winter of 1953—54 the new virus was preserved in live plants, in 1954, 55 and subsequent years it was kept in dry leaves. After carrying out the appropriate tests, which will be published, the new virus was identified as a new strain of the tobacco mosaic virus. It was called S-strain of tobacco mosaic virus from the stripe symptoms which it forms on tobacco leaves.

Discussion

The symptoms of yellow and green spots on leaves of the medium level cannot be regarded as a sign of a reversal to the original Al-strain, because:

1. Reinoculation with sap from the tissues of these parts always produced the typical symptom picture for the S-strain and isolation from individual spots and from lesions did not lead to the selecting out of the Al-strain.

2. The only common symptoms with the Al-strain are these patches and they sometimes appear only temporarily. With the S-strain, however, deformation of the blade and albinism is completely lacking (as distinct from the Al-strain). With the Al-strain the youngest leaves are commonly very strongly affected, while the opposite is the case for the S-strain, the youngest leaves usually remain unaffected.

The symptoms are also reproduced without change in passages through plants from a normal glass house environment followed by passages through plants on agar if it is carried out by selection of sap from tissues with different symptoms (with yellow or green stripes, with yellow or green spots and rings); thus not even this method produced a new form. The stylisation used by Svobodová in 1954, when the occurrence of these spots gave rise to uncertainty as to whether a permanent change is involved, is now therefore made precise. The permanency of these symptoms is further confirmed by the fact that in four reproductions of the origin of the S-strain no symptomatic differences were observed, although the different reproductions were carried out at different times of the year. The results were also the same whether sap from tomatoes or from tobacco was used as the original material.

As shown by the passage of the virus through dwarf plants on micro-beds in a free environment (SVOBODOVÁ 1954), the changed metabolism of the dwarfs did not suffice to produce the change from Al-strain to S-strain; it was necessary to influence the metabolism by an abnormal environment in Erlenmeyer flasks after inoculation. The reduced metabolism of the dwarfs inhibited the development of the virus, but did not change it qualitatively.

It was also found that neither the passage of the virus to dwarf plants on micro-beds nor on agar sufficed in itself to produce the change. Here also there was only a weakening amounting almost to disappearance of the symptoms. A twofold change in the environment of the virus was necessary to achieve the symptoms described: first of all passage from normal plants to dwarfs and then from dwarfs back to normal, where the virus once again had normal conditions for reproduction. In the subsequent (third) passage to normal plants the change became apparent. The case was similar when a plant infected with Al-strain and grown on agar was transplanted and grown further in normal conditions. In the subsequent passage (again the third) through plants grown on agar, the same change occurred. The symptoms of S-strain appeared in the third passage without regard to whether the plants used were grown in garden soil in the normal way, or were dwarfed plants; but before this the virus had to undergo at least twice the change of host environment described.

In the work of PFANCHUCH et al. (1940) the strain TM44 is described, which

according to the symptoms is in some respects similar to the S-strain. It was developed from an ordinary green strain of tobacco mosaic through X-ray irradiation of the host plant. The lighter colouring of the tissues along the veins is, however, described in the case of TM44 as a secondary symptom; with the S-strain it appears as the first symptom on the inoculated leaves. The remaining symptoms are quite different. The incubation period for the TM44 type disease is, similarly to that for the S-strain, longer than for ordinary tobacco mosaic virus. The pathogenicity of TM44 was, however, increased as compared with the initial ordinary strain, while that of S-strain was lower as compared with both the Al-strain and the ordinary green strain. An increased variability in the expression of the symptoms in relation to conditions of illumination was also observed in both cases. It would seem that the influence of the X-rays on the metabolism of the host produced a similar reaction to that of a brief period of cultivation of the virus in dwarfed plants in which the reproduction of the virus over a certain period was limited.

According to the opinion of KAUSCHE et al. (1940), mutation is not to be regarded as a direct change of virus molecule induced by an influencing factor (a changed substratum) but it appears in the course of the reproduction of the virus molecule in a changed substratum, provided that the synthesis of proteins in an irradiated organism is not inhibited by the stoppage in the total growth. Similarly, no change occurred in our work either in the dwarfed plants in which growth was not completely stopped, (as by X-ray irradiation), but greatly inhibited. The change in symptoms did not occur until after passage into normal plants, that is, as with KAUSCHE, when the reproduction of the virus was not being inhibited by the arrested growth of the host plant. These plants, in contrast to irradiated plants, were absolutely unaffected.

In most of the work on the origin of new strains a dominant question is whether the new strain has arisen by mutation or by selection (e. g. KASSANIS 1957). Recently an increasing number of authors have been producing evidence of the bringing about of mutation. For example, MUNDY (1957), after observing all possible precautions in his work, comes to the conclusion that in the change from green to yellow strains and the reverse, achieved under the influence of changed temperatures of the leaves, it is not a matter of selection but of true mutation. The TM44, above mentioned, is also a result of a mutation. The aim of this work was to establish whether a change in the metabolism of a host plant of the same species and variety through a change in its environment is sufficient to call forth an alteration in the properties of the virus. The question whether this change in properties is due to mutation or selection has been left aside, although for a number of reasons we consider the possibility of selection to be less probable in our case. The strong dependence of the virus on the metabolism of the host, the evidence for which becomes clearer and clearer, is in our opinion a reason for the tendency to variability of the properties of the virus.

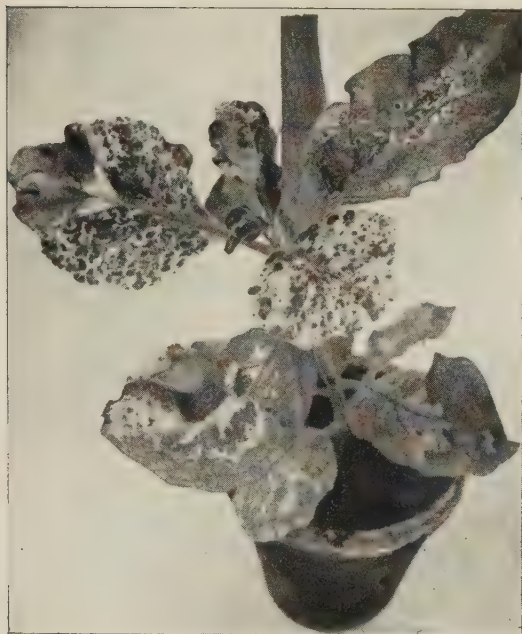


Fig. 1.

Fig. 1. *Nicotiana tabacum* Samsun, systemically diseased with A1-strain. The sap from this plant was the original material for passing, which led to the development of the new strain (S-strain).

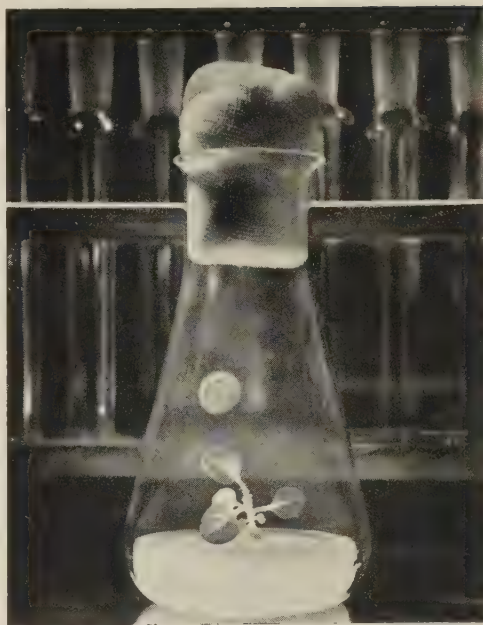


Fig. 2.

Fig. 2. Dwarfed plant of *Nicotiana tabacum* Samsun, 3½ months old (118 days), before infection. The plant is forming aerial roots.



Fig. 3.

Fig. 3. *Nicotiana tabacum* Samsun plant diseased with the S-strain of TMV. Yellow stripes along the veins of the leaf next above the inoculated leaf.



Fig. 4.

Fig. 4. *Nicotiana tabacum* Samsun plants diseased with the S-strain of TMV. Yellow stripes along the veins pass over on the further leaves to green stripes.

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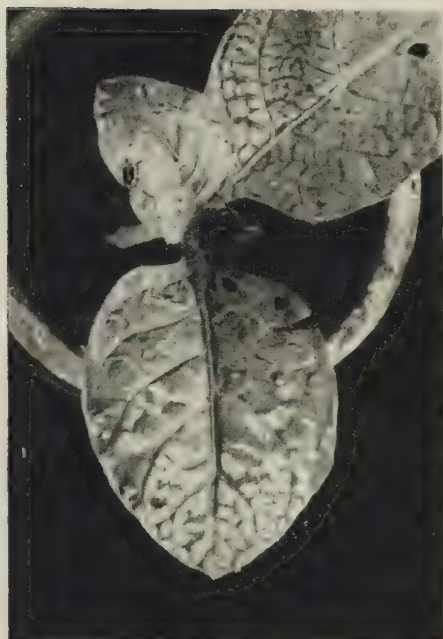


Fig. 5.

Fig. 5. *Nicotiana tabacum* Samsun. In the further course of the S-strain disease spots, which under favourable conditions of illumination and temperature are bordered by bands, are formed beside the green stripes along the veins.



Fig. 6.

Fig. 6. Stripe patterns on older leaves of fully grown *Nicotiana tabacum* Samsun plants diseased with the S-strain of TMV.



Fig. 7.

Fig. 7. Yellow stripes along the veins of a *Nicotiana tabacum* Samsun plant on agar medium in an Erlenmeyer flask (S-strain TMV).



Fig. 8.

Fig. 8. Green stripes along the veins of a *Nicotiana tabacum* Samsun plant on agar medium (S-strain TMV).

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Возникновение нового штамма табачной мозаики в результате воздействия на метаболизм хозяина

ЯРМИЛА СВОБодОВА

Резюме

Описывается новый штамм вируса табачной мозаики, который был назван S-штаммом ВТМ. В качестве исходного материала использовался А1-штамм ВТМ.

В отличие от применявшихся до сих пор методов S-штамм был получен в результате прямого вмешательства в метаболизм растения-хозяина путем изменения его условий выращивания. В опыт были взяты обычные хозяева ВТМ, — прежде всего растения табака и томатов, которые выращивались в асептических условиях в колбах Эрленмайера на среде с агаром при понижении количества питательных веществ, сокращении светового дня и ограничении доступа воздуха, так что растения остались карликовыми. После пассажа исходного штамма ВТМ (А1-штамма) этими карликовыми растениями наблюдалось изменение признаков, которое проявилось только при дальнейших пассажах на растениях, выращенных в нормальных условиях в садовой почве в теплице, где вирус опять получил возможность нормально размножаться. Из развившихся признаков далее исследовалось только наиболее выразительное изменение (признак полосок S-штамма). В результате этих попеременных пассажей А1-штамма ВТМ нормальными растениями и карликовыми, выращенными в искусственной питательной среде, — вот, что условия выращивания вируса, были подвержены двух — или трехкратному резкому изменению, — был получен новый штамм (S-штамм) — пять раз в различное время года. Что касается симптомов, разницы между отдельными воспроизведениями этого штамма не обнаружилось. Результаты получались одинаковыми независимо от того, был ли исходный материал взят из томатов или из табака сорта Samsun.

На возникновение нового штамма повлияла не только карликовость растений-хозяев: как показали пассажи вируса карликовыми растениями на микрографиках на свободном воздухе, для его возникновения было необходимо воздействие на метаболизм ненормальной среды (прежде всего пониженный обмен воздуха в колбах Эрленмайера) еще после инокуляции.

После обратных пассажей S-штамма с растений, выращенных обычным способом в теплице, на карликовые растения в искусственной питательной среде дальнейших изменений уже не наблюдалось. И пассажи на 10 других хозяевах, выращенных обычным способом в садовой почве или же в искусственной питательной среде, уже не вызвали никаких дальнейших изменений признаков. S-штамм поддерживается постоянными пассажами на *Nicotiana tabacum* Samsun в течение 6 лет, причем каждый год производится массовая инокуляция большого количества растений. Поэтому мы считаем полученное изменение устойчивым.

Исследования биологических, химических и других особенностей S-штамма и их сравнение со свойствами его исходного штамма и обыкновенного зеленого штамма ВТМ будут опубликованы.

Для возникновения нового штамма было достаточно косвенного влияния на вирус посредством изменения метаболизма растения-хозяина, что подтверждает зависимость качеств вируса от метаболизма хозяина. Изменение проявляется только после пассажа нормальными растениями неподверженными никакому влиянию, когда репродукция вируса уже не была торможена замедленным ростом растения-хозяина. Возникновение S-штамма аналогично с возникновением ТМ44-штамма, который получил Pfaffbuch и д. под влиянием X-лучей. В качестве исходного материала этот автор использовал обыкновенный зеленый штамм табачной мозаики.

Root Formation in Plants Infected with Yellows-type Viruses

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Souhrn

Byl sledován vliv infekce stolburem a čs. metlovitostí brambor typu II na tvorbu kořenů u řízků rajčat. Obě choroby potlačují signifikantně tvorbu kořenů, jsou-li jejich symptomy plně vyvinuty. Metlovitost měla výraznější vliv než stolbur. Infekce těmito žloutenkovými viry také značně zvyšuje variabilitu ve váze kořenů.

Summary. Root formation was studied in cuttings from tomato plants infected with stolbur and Czechoslovak type II potato witches' broom viruses. A highly significant difference in root formation was always found in plants with clear-cut disease symptoms. Potato witches' broom virus inhibited the root growth more than stolbur. In all cases, diseased plants showed a greater variability in the weights of roots than healthy controls.

Plant viruses very frequently affect, and in the majority of cases inhibit, growth of the infected plants. Yellows-type viruses very often cause marked morphological changes in various plant organs. It could, therefore, be assumed that in such cases the changes in metabolism of growth substances would be much more deep-seated than in other virus infections.

Investigations were carried out into the effect of infection with two yellows-type viruses, stolbur and potato witches' broom, on root formation, which is known to depend strongly on growth substances.

Tomato plants (*Solanum lycopersicum* L., variety Champion) were inoculated by grafting at the 6—7 leaf stage. At certain intervals after inoculation, shoots with about 4 fully developed leaves were cut off and fixed individually in

Table 1. Root formation in tomato plants infected with yellows-type viruses

			n	$\bar{x} \pm 3 \cdot s_{\bar{x}} \text{ mg. } \%$	$\bar{x} \pm 3 \cdot s_{\bar{x}} \%$	v %	t(N)	p
1.	H ₂ O	K	20	254 $\pm$ 3.20	100 $\pm$ 3.7.9	35	—	—
		S	21	238 $\pm$ 3.28	94 $\pm$ 3.11.0	35	0.46	n
		WB	18	120 $\pm$ 3.23	47 $\pm$ 3.9.1	86	4.35	b
	IAA	K	21	550 $\pm$ 3.39	100 $\pm$ 3.7.1	33	—	—
		S	21	488 $\pm$ 3.55	89 $\pm$ 3.10.0	51	0.95	n
		WB	19	167 $\pm$ 3.26	30 $\pm$ 3.4.7	67	8.23	b
2.	H ₂ O	K	20	361 $\pm$ 3.45	100 $\pm$ 3.12.5	56	—	—
		S	20	198 $\pm$ 3.41	55 $\pm$ 3.11.4	93	2.68	a
		WB	20	84 $\pm$ 3.22	23 $\pm$ 3.6.1	119	5.47	b
	IAA	K	20	384 $\pm$ 3.30	100 $\pm$ 3.7.8	35	—	—
		S	20	219 $\pm$ 3.29	57 $\pm$ 3.7.5	59	3.98	b
		WB	20	103 $\pm$ 3.19	26 $\pm$ 3.5.0	83	7.95	b
3.	H ₂ O	K	20	411 $\pm$ 3.14	100 $\pm$ 3.3.4	15	—	—
		S	19	181 $\pm$ 3.22	44 $\pm$ 3.5.4	53	8.85	b
	IAA	K	20	435 $\pm$ 3.44	100 $\pm$ 3.10.1	48	—	—
		S	20	95 $\pm$ 3.21	22 $\pm$ 3.4.8	101	6.63	b
4.	H ₂ O	K	19	185 $\pm$ 3.22	100 $\pm$ 3.11.9	53	—	—
		S	20	27 $\pm$ 3.8	15 $\pm$ 3.2.1	137	6.62	b
	IAA	K	20	163 $\pm$ 3.13	100 $\pm$ 3.8.0	36	—	—
		S	20	16 $\pm$ 3.5	10 $\pm$ 3.3.1	156	10.29	b

IAA, H₂O variations of experiment with and without treatment with indoleacetic acid; K, S, WB, healthy controls, plants infected with stolbur and potato witches' broom; **n** number of cases; $\bar{x}$ median; $s_{\bar{x}}$ median error; **v** Pearson's coefficient of variability; **p** significance of the difference (after Fisher's tables of *t* values); **n** difference not significant (over 5% limit of significance), **a** difference highly significant (1%—0.1% limit of significance), **b** difference highly significant (over 0.1% limit of significance); *t*(N) value of *t* criterion for respective number of degrees of freedom

$$N = n_1 + n_2 - 2$$

$$t(N) = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s_{x_1}^2 + s_{x_2}^2}}$$

ROOT FORMATION IN YELLOWS INFECTED PLANTS



Fig. 1—3. Tomato cuttings from healthy controls (1), from plants infected with stolbur (2) and potato witches' broom (3) from experiment 2. Cuttings with minimum, average and maximum root formation are shown. Photograph by Valenta.

openings of plastic plates, which were placed on the top of five litre glass vessels, twenty shoots in each vessel. Rooting was carried out in regularly changed tap water in the light at room temperature. Each experiment had two variations: in the first the shoots were placed directly in water, in the second they were treated with 10^{-4} M. solution of indoleacetic acid for 24 hours. About 2 cm. of the shoots were immersed into the water or IAA solution. After a fortnight the roots were cut off with a scalpel from each shoot, lightly dried with filter paper and weighed.

In experiments 1 and 2 stolbur virus strain Čs 7/55 of medium virulence, and Czechoslovak type II potato witches' broom virus strain Čs1/55 (VALENTA 1958) were used. In experiments 3 and 4 stolbur virus strain Čs 9/55, also of medium virulence, was used. The plants for the first two experiments were grafted on April 21, 1956, and the shoots for the first and second experiments were cut off 30 and 63 days after grafting, respectively. In the first case only some of the stolbur infected plants showed slight symptoms, others were without symptoms, and most of the witches' broom infected plants had clear-cut symptoms. In all the other experiments the plants showed fully developed disease symptoms. For experiments 3 and 4 the plants were grafted on June 27, 1957, and shoots for rooting were cut off 43 and 105 days later, respectively.

In plants with developed disease symptoms a highly significant difference in root formation was always found. Witches' broom inhibited root growth more than stolbur (Tab. 1, Fig. 1—3). In all cases the infected plants showed an increased variability of root formation, expressed by Pearson's coefficient of variability v , as compared with healthy plants.

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Корнеобразование у растений зараженных вирусами типа желтух

ЯН ЗАВАДА и ВЛК ВАЛЕНТА

Резюме

Исследовалось образование корней у черенков из растений томата, зараженных вирусами столбура и чехословацкого типа II ведьминой метлы картофеля. У растений с выраженными признаками заболевания всегда были обнаружены значительные различия корнеобразования. Ведьмина метла картофеля подавляла рост больше чем столбур. Во всех случаях, изменчивость корнеобразования у больных растений была выше чем у здоровых контрольных растений.

The Behaviour of the S-strain of Tobacco Mosaic Virus as Determined by Biological Tests

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Souhrn

Byly popsány příznaky, které působí nově vzniklý S-kmen VTM (SVOBODOVÁ 1954, 1958, 1959) u deseti hostitelů a porovnány se symptomy, které vytváří Al-kmen a obyčejný zelený kmen tabákové mosaiky. S-kmen vytváří na všech zkoušených hostitelských rostlinách příznaky od ostatních kmenů odlišné. Srovnání inkubační doby a ovlivnění růstu na *Nicotiana tabacum* Samsun, chování na *N. rustica* a kvantitativní testy na *N. glutinosa* ukázaly, že S-kmen je ze všech srovnávaných kmenů nejméně patogenní. Podle výsledků všech biologických testů je S-kmen bližší obyčejnému kmenu tabákové mosaiky; pouze sérologické a interferenční zkoušky svědčí o jeho bližším příbuzenství k Al-kmenu, ze kterého vznikl. Chování S-kmene VMT ve vytlačené šťávě je shodné s chováním obyčejného zeleného kmene i Al-kmene. Stejně tak okruh hostitelů všech tří kmenů je týž. Diferenčním hostitelem do určité míry je *Physalis alkekengi* a *P. Franchetti*. Podle biologických testů je tedy S-kmen novým kmenem viru tabákové mosaiky.

Summary

1. The symptoms caused by the S-strain (SVOBODOVÁ 1954, 1958, 1959) were described for ten hosts and compared with those caused by the Al-strain and the ordinary green strain of the tobacco mosaic virus. In all the host plants tested the S-strain produces symptoms different from those caused by other strains.

2. A comparison of the incubation period and the effect on growth of *Nicotiana tabacum* Samsun, the behaviour on *N. rustica* and quantitative tests on *N. glutinosa* showed that the S-strain is the least pathogenic of all the strains compared.

3. Results of biological tests show the S-strain to be most similar to the ordinary green strain of tobacco mosaic virus, only serological and cross-protection tests indicate a closer relationship to the Al-strain, from which it developed.

4. The behaviour of the S-strain of TMV in expressed sap is similar to the behaviour of the ordinary green strain and of the Al-strain. The range of hosts for all three strains is also the same. *Physalis alkekengi* and *P. Franchetti* are to a certain extent differential hosts.

Thus biological tests show that the S-strain is a new strain of tobacco mosaic virus.

Introduction

The S-strain of tobacco mosaic was obtained by influencing the metabolism of the usual host plants of this virus. It developed by the passaging of the Al-strain of TMV on the same varieties of tobacco and tomato plants. The passaging was carried out alternately on normal and stunted plants which had been cultivated under aseptic conditions on an agar medium in Erlenmeyer flasks with limited air supply. It was after two or three changes of the environment of the virus brought about by transfer from a plant with normal metabolism to a plant with altered metabolism and the reverse (Svobodová 1954, 1958, 1959), that this strain was obtained. The new strain has now been maintained by continual passaging for six years. The symptoms it causes clearly distinguish it from all other available strains. Its biological characteristics have been ascertained in comparison with the characteristics of its parent strain (Al-strain of TMV) and of the ordinary green strain of tobacco mosaic virus.

Methods

The following hosts were chosen for establishing and comparing the relationship of the new strain to its parent strain (Al-strain) and the ordinary green strain of TMV: *Nicotiana tabacum* Samsun, *N. tabacum* White Burley, *N. tabacum* Virginian golden, *N. glutinosa*, *N. rustica*, *Physalis Franchetti*, *P. alkekengi*, *Capsicum annuum*, *Solanum lycopersicum*. The symptoms produced by systemic infection of leaves were also described for these hosts.

For *Nicotiana tabacum* Samsun the length of the incubation period for the different strains was compared and their effect on the growth of the infected plants was observed. In order to evaluate biological activity, a qualitative and quantitative comparison of primary lesions caused by inoculation of the leaves of *N. rustica* was also made and the relative infectivity of homogenates of systemically diseased plants was compared by testing on *Nicotiana glutinosa*. The mutual relationship of the new strain with the two other strains was further determined serologically. (The drop method according to JERMOLJEV and HRUŠKA, 1947 and BAWDEN, 1950). The identification of the new strain (S-strain) was completed by determination of the limiting inactivation temperature of the virus in sap and by achieving infection from dried material.

Results

1. Review of symptoms caused by individual strains on different host plants is given in the table 1.

On all the host plants mentioned the S-strain produces symptoms which differ both from the symptoms caused by the Al-strain and the ordinary strain, and from those of all other strains. On all host plants these symptoms

Table 1.

Host plant	Ordinary green strain of TMV	Al-strain of TMV	S-strain of TMV
1. <i>Nicotiana tabacum</i> Samsun	Typical tobacco mosaic; green spots deformation of leaves.	Dark-green, whitish, yellow, yellow-green spots, blisters, deformation. Youngest leaves with strong symptoms. Some plants die, majority stop growing.	At first yellow stripes along veins, later leaves with green stripes, yellow and green spots, finally stripe patterns. Youngest leaves, at least their apexes, without symptoms. Growth almost completely non-inhibited.
2. <i>N. tabacum</i> White Burley	The same symptoms as <i>N. t.</i> Samsun.	The same symptoms as those caused by Al-strain on <i>N. t.</i> Samsun.	The same symptoms as those caused by S-strain on <i>N. t.</i> Samsun, but weaker.
3. <i>N. tabacum</i> Virginian Golden	The same symptoms as <i>N. t.</i> Samsun	The same symptoms as those caused by Al-strain on <i>N. t.</i> Samsun	The same symptoms as those caused by S-strain on <i>N. t.</i> Samsun; the reaction of this variety is more sensitive; decolouration symptoms are clearer at a time of year when symptoms on Samsun are not yet evident.
4. <i>N. glutinosa</i>	Primary necrotic lesions.	Primary necrotic lesions.	Primary necrotic lesions.
5. <i>N. rustica</i>	Slight lesions, often with yellowish surroundings. Leaves with many lesions often go completely yellow. Sometimes large areas of necrosis. Frequent large yellow spots on younger leaves.	Slight lesions, larger areas of necrosis.	Slight lesions.

Tab. 1. *contin.*

Host plant	Ordinary green strain of TMV	Al-strain of TMV	S-strain of TMV
6. <i>Physalis Franchetti</i> and 7. <i>P. alkekengi</i>	No symptoms.	Dark-green whitish, yellow, yellow-green spots, blisters, deformations. Youngest leaves show strong symptoms. Some plants die, some stop growing.	Most often without symptoms, or slight clearing of veins, light yellowish decolouration, youngest leaves stay green.
8. <i>Capsicum annuum</i>	Not determined.	Following infection of cotyledons; cotyledons, go yellow and plants die. Older plants have yellowish spots.	The same symptoms, but considerably milder.
9. <i>Solanum lycopersicum</i>	Not determined.	Dark-green, whitish, and yellow spots, blisters and deformations.	Yellow spots, rarely green blisters; disease slight.

are very mild. As far as pathogenity, judged only by symptoms, is concerned, the S-strain resembles the ordinary tobacco mosaic more closely than its parent strain. It is, however, the mildest of all three strains.

2. The range of host plants

As far as plants entered in the above table are concerned, all three strains have the same range of host plants. Only *Physalis Franchetti* and *P. alkekengi* form an exception as far as the symptoms of disease are concerned. Following one infection with ordinary tobacco mosaic they show no symptoms; in order to produce symptoms it is necessary to repeat infection of the same plant on several consecutive days (BLATTNÝ 1958). The Al-strain attacks this plant very strongly. The S-strain produces mild symptoms, which are sometimes not very pronounced.

3. Comparison of the incubation period and the effect on the growth of plants of *Nicotiana tabacum* Samsun

The comparison was carried out on plants of *Nicotiana tabacum* Samsun at the stage when they had 4—5 leaves. The plants were infected with the three strains. There were 36 plants in each group. (The results are given here for information only in order to fill in the results of the other experiments in a general way.)

The first symptoms began to appear in plants infected with the Al-strain and only at a later stage and less clearly in plants infected with the other strains. Then, 7 days after infection, symptoms were observed as follows:

in 83.3% of plants infected with Al-strain, in 63.8% of plants infected with the ordinary green strain and in 38.8% of plants infected with the S-strain. After a further 24 hours definite symptoms were observed in 94.4% of plants infected with the Al-strain, in 94.4% of plants infected with the ordinary green strain and in 66.6% of plants infected with the S-strain. During the following days symptoms of systemic infection were shown by all plants infected with the Al-strain and the ordinary green strain. In the group infected with the S-strain one plant remained without any signs of disease. The average height one month after infection was for plants infected with the ordinary green strain 7.6 cm., for those infected with the S-strain 6.6 cm. and for those infected with the Al-strain 3.6 cm.

With regard to the incubation period and the effect on the growth of the host plant it appears that the S-strain is more similar to the ordinary strain of tobacco mosaic virus than to its parent strain. Of all the strains compared it is, however, the mildest in its effect on the host plant.

4. Behaviour on *Nicotiana rustica*

Groups of 5 plants with 4 leaves were infected with each of the strains under comparison. The third leaf was infected with centrifuged, filtered sap by means of a brush. Fifty-two days after the date of infection (the beginning of November) the average number of lesions on an inoculated leaf was 40.2 for the ordinary green strain, 10.6 for the Al-strain and 8.6 for the S-strain. The average number of leaves showing signs of systemic infection was 2.4 for the ordinary green strain, 1.8 for Al-strain and 1.2 for the S-strain. The primary reaction to the ordinary green strain and to the Al-strain was accompanied by several large areas of necrosis and chlorosis, while in the primary reaction *Nicotiana rustica* infected with the S-strain showed only slight lesions.

Thus the S-strain is seen to be the least pathogenic for plants. Similarly, with regard to its effect on growth S-strain proved to be the least harmful. Plants infected with S-strain reached an average height of 10.9 cm. (while plants infected with the green strain attained 9.8 cm. and those infected with the Al-strain only 8.8 cm.); the average number of leaves per plant was also highest with the S-strain, i. e. 6.4, for the green strain it was only 5.6 and for the Al-strain 5.2. These results are given for information. In the spring and summer the differences between these strains are more pronounced.

5. Quantitative tests on *Nicotiana glutinosa*

Two quantitative tests were carried out. For the first time, in September 1956, *Nicotiana tabacum* Samsun plants were used, which had been infected 33 days previously and were of equal age and had been cultivated under the same conditions. Sap was in every case taken from only one plant of those infected with each strain. After filtration and centrifuging this sap was inoculated without dilution on to *Nicotiana glutinosa* by painting the leaves with a brush, one half of each inoculated leaf blade being painted with sap from plants infected with the S-strain and, in the first group, the other half

with sap from plants infected by the Al-strain, in the second group with sap from plants infected by the ordinary green strain. In each group 12 leaves were tested in this way. The sap was not washed off the leaves.

The results of this test are in agreement with those of a test carried out in May of the following year (1957), in which plants of *Nicotiana tabacum* Samsun infected 21 days previously were used. The leaves with petioles were cut and twice the amount by volume of distilled water was added to the amount by weight of the leaves taken from each plant separately. Following homogenization the expressed sap was refiltered and centrifuged. Inoculation was carried out as in the first test. Groups of twenty leaves of *Nicotiana glutinosa* were used for testing. Results of both tests are given in the following table:

Quantitative test on *Nicotiana glutinosa*

Table 2. Average number of lesions on one leaf:

Date	S — Z		S — Al		Al — Z		n
14. 9. 1956	49.3	83.9	62.9	146.7			12
27. 5. 1957	9.1	19.3	7.7	36.1	41.3	33.2	20
Average	24.2	43.5	26.4	71.2			

Statistical evaluation (by t-test) was carried out for the experiment of 14. 9. 1956 with a lower n (12) and here there were also smaller differences in averages than in the further comparative tests. In the comparison of S-strain with Al-strain there are statistically significant differences ($P < 0.01$), in the comparison of S and Z the result is somewhat below the limits of significance ($t_{22} = 1.902$, $P < 0.10 > 0.05$).

It may be said that the least biological activity was shown by the S-strain: the ordinary green strain of TMV showed a medium activity and the Al-strain showed the highest activity. Thus the S-strain more closely resembles the ordinary strain in its infectivity than the Al-strain.

6. Cross-inoculation

Nicotiana tabacum Samsun was used for cross-inoculation. The plants were infected with the second strain in each case three days after infection with the first, that is sooner than any symptoms caused by the first strain could appear. It was found that the S-strain protects tobacco plants against secondary infection by the Al-strain, and alternatively that the Al-strain protects against the S-strain. These results are in agreement with BLATTNÝ'S (1956) results and indicate a close relationship between the S-strain and the Al-strain and a less pronounced similarity between these two strains on the one hand and the green strain on the other hand.

7. Serological tests

Two tests of serological relationship were carried out: antigen was similarly prepared for both tests. The virus was isolated according to SIEGEL and WILDMAN's method (1954). Further purification was then carried out by three-fold coagulation at the isoelectric point according to BAWDEN and PIRIE (1943). Following dialysis the antigen was filtered through a bacterial filter G5; for the second test lyophilized purificate was used. Immunization was carried out as follows: on the first day rabbits were injected with 1 ml. each of antigen, on the second day 1.5 ml., the fifth day 2 ml., the sixth day 2.5 ml., the seventh day 3 ml., the ninth day 3.5 ml., the tenth day 4 ml. After an interval of one week the rabbits were starved for one day and were then bled. The serum, after centrifuging, was put into ampules at a temperature of -10°C .

In 1956 serological tests were carried out by the drop method according to JERMOLJEV and HRUŠKA (1947) and in 1957 by the classic method (in test tubes) according to BAWDEN (1950). The different virus preparations were diluted with physiological saline in geometric series. The strength of the reaction was judged according to the dilution in which the virus preparation being tested still reacted with the appropriate serum.

In the first and second tests the serum against the S-strain reacted more strongly with the virus preparation of the Al-strain than with that of ordinary tobacco mosaic virus. The serum against the ordinary tobacco mosaic virus reacted more strongly with the Al-strain than with the virus preparation of the S-strain. It can therefore be concluded from the results that all the strains are serologically very closely related; a particularly close relationship exists between the S-strain and the Al-strain; and also between Al-strain and the ordinary strain. However, only small differences are involved. The results of the serological tests are in agreement with those of the protection tests.

8. The behaviour of the strains under comparison in expressed sap

The S-strain, similarly to the Al-strain and the ordinary green strain of tobacco mosaic virus, can stand a temperature of 90°C for a period of 10 min. without losing its activity. It keeps its infectivity in dried sap and dry leaves. Thus it behaves in the same way as the other two strains.

Discussion

The S-strain is most closely related to the ordinary green strain of tobacco mosaic virus both in its pathogenity and its behaviour in the host plant. On the other hand, interference and serological tests indicate a closer relationship to the Al-strain from which the S-strain originated. The similarity to the ordinary green strain of tobacco mosaic is particularly remarkable in view of the fact that KAUSCHE and STUBBE's strain TM44, which the S-strain resembles in certain respects (SVOBODOVÁ 1958, 1959), arose by mutation

directly from the green strain of ordinary tobacco mosaic. TM44, however, acts more pathogenically than the ordinary strain of TMV, while the S-strain is the least pathogenic of all the strains which have been compared. Although both these strains produce similar symptoms, their pathogenesis is diametrically opposed. This indicates some kind of mutual independence in the production of symptoms by the virus and by the plant. This observation coincides to a certain extent with the occurrence of a large quantity of virus in carriers which do not show symptoms.

The writer wishes to express her thanks to Dr. Brčák for his help in connection with the quantitative tests on *N. glutinosa*, to Ing. Pozděna for help with the serological tests and to J. Limberk for help with the tests on *N. tabacum* Samsun.

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Поведение S-штамма ВТМ, установленное биологическими методами

ЯРМИЛА СВОБODOVÁ

Резюме

1. Описаны признаки, вызываемые S-штаммом (Svobodová 1954, 1958, 1959) у десяти растений-хозяев и сопоставлены симптомы, вызываемые А1-штаммом и обычным зеленым штаммом вируса табачной мозаики. S-штамм образует на всех подопытных растениях-хозяевах признаки, отличающиеся от других штаммов.

2. Сравнение времени инкубации и влияние на рост на *Nicotiana tabacum* Samsun, поведение на *N. rustica* и количественные тесты на *N. glutinosa* показали, что S-штамм обладает самой низкой патогенностью среди сравниваемых штаммов.

3. По результатам биологических тестов S-штамм ближе обыкновенному зеленому штамму табачной мозаики; только серологические и интерференционные опыты свидетельствуют о его близком родстве с А1-штаммом, из которого возник.

4. Поведение S-штамма ВТМ в отпрессованном соке соответствует поведению обычного зеленого и А1-штамма. Тот же диапазон растений-хозяев всех трех штаммов тот же. Дифференцирующим хозяином до определенной степени является *Physalis alkekengi* и *P. Franchetii*.

По результатам биохимических тестов S-штамм является новым штаммом вируса табачной мозаики.

Cadmium Ions as Inhibitors of Tobacco Mosaic Virus

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Souhrn

Účinek kademnatých iontů na reprodukci viru tabákové mosaiky byl studován na rostlinách tabáku (*N. tabacum* Samsun), které byly pěstovány ve skleníku v kořenáčích. Pokusné rostliny byly zalévány roztokem síranu kademnatého o koncentraci 4 mg Cd^{2+} /l ml v různých dávkách. U každé rostliny byl očkován jeden list Al-kmenem VTM. Optimální dávka kademnatých iontů, která nemá žádný škodlivý vliv na pokusné rostliny, je dávka 160 mg Cd^{2+} na jednu rostlinu aplikovaná ve dvou zálivkách, a to těsně před inokulací a třetí den po inokulaci. Reprodukce VTM je inhibována ze 70 % podle testu na *N. glutinosa* a hypersensitivním hybridu. Inhibiční účinnost kademnatých iontů značně poklesne při zalívce roztokem kademnatých iontů 24 hodiny před inokulací. Z rozdílů v průběhu primární infekce, sledované jodovou zkouškou, jsou nápadné zvláště tyto skutečnosti: u pokusných rostlin se ztrácí škrob z infikovaných listů pomaleji, vývoj chlorotických skvrn je zbrzděn a jejich ohraničení a struktura nejsou ostré. Vyšší dávky kadmia (kolem 300 mg na jednu rostlinu) vyvolávají na některých listech mladých rostlin tabáku (*N. tabacum* Samsun) skvrny, které připomínají nekrotické lese na listech *N. glutinosa* po infekci VTM. Předběžně bylo zjištěno, že kademnaté ionty vyvolávají poruchy v metabolismu fosforu. V diskusi je vyslovena domněnka, že inhibiční účinek kademnatých iontů na reprodukci VTM může spočívat, analogicky k představě vytvořené v živočišné fyziologii, v interakci iontů zinku a kadmia v metabolismu nukleoproteinů.

Summary

1. While studying the inhibitory effects of cadmium ions on the reproduction of tobacco mosaic virus in vivo it was found that a dose of 160 mg. Cd^{2+} applied in two waterings to one plant (immediately before inoculation and

the third day following inoculation) produced an average 70% inhibition, without in any way injuring the plants themselves.

2. The inhibitory effect of cadmium ions decreased considerably when the plants were watered with a cadmium ion solution 24 hours before inoculation.

3. The differences in the course of primary infection, as followed by means of iodine tests, were particularly apparent in the following respects: starch is lost from the infected leaves of the experimental plants more slowly, the development of chlorotic spots is slowed down and their delimitation and structure are not so sharp.

4. On some leaves of young tobacco plants (*N. tabacum* Samsun) larger doses of cadmium produce spots, which resemble the necrotic lesions on leaves of *N. glutinosa* following infection with TMV.

5. Preliminary determination indicates that cadmium ions cause a disturbance of phosphorus metabolism.

6. In the discussion it is suggested that the inhibitory effect of cadmium ions on the reproduction of TMV may, analogically to the conception existing in animal physiology, be connected with the interaction of zinc and cadmium ions in nucleoprotein metabolism.

Introduction

The effects of many different substances on the reproduction of plant viruses have been the subject of much work, which has been summarized for instance by MATHEWS and SMITH (1955). Recently the greatest attention has been devoted to derivatives of purines and pyrimidines, which are also the only substances for which the mechanism of the inhibitory effect has been explained (MATHEWS and SMITH 1955). These derivatives block the biosynthesis of virus nucleic acids by becoming incorporated into the molecules in place of the natural purines and pyrimidines.

Of the series of metal ions which have been tested as agents against plant viruses (e. g. MATHEWS and SMITH 1955, RYZHKOV and MARCHENKO 1957), the most significant inhibition was found with zinc (STODDARD 1947, WEINTRAUB et. al. 1952, RUMEX and THOMAS 1951). The results are not, however, quite consistent. YARWOOD (1953, 1954) came to the opposite conclusion and demonstrated the stimulatory effect of zinc on the reproduction of tobacco mosaic virus. This contradiction was explained by HELMS and POUND's work (1955); they found that zinc affects the reproduction of tobacco mosaic virus in the same measure as the growth of the host. According to these authors, increased doses of zinc increase virus accumulation insofar as they stimulate the growth of the host.

The aim of the present work has been to find an effective chemotherapeutic agent, the application of which would, at a later stage of this work, make it possible to examine the mechanism of virus nucleoprotein biosynthesis. From the start the use of purine and pyrimidine derivatives was excluded, since these are substances the mode of action of which is known. On the basis of

CADMIUM IONS AS INHIBITORS OF TOBACCO MOSAIC VIRUS



Fig. 1a



Fig. 1b

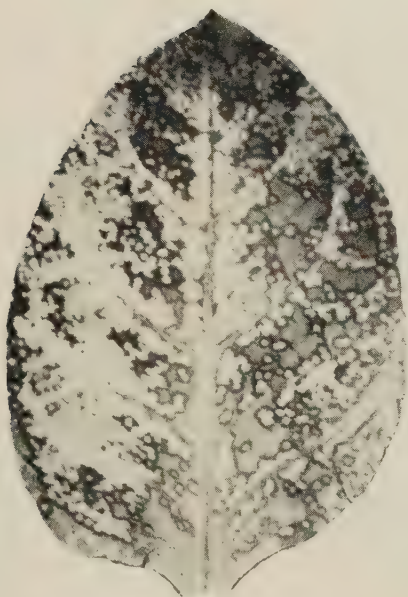


Fig. 2a



Fig. 2b

Course of primary infection, followed by the iodine test, on leaves of *N. tabacum* Samsun after inoculation with A1-strain TMV and watering with cadmium sulphate solution (dose 160 mg. Cd^{2+} per plant) divided between two waterings: immediately before inoculation and third day after inoculation.

Fig. 1. 3rd day after inoculation. a = control, b = experiment.

Fig. 2. 4th day after inoculation. a = control, b = experiment.

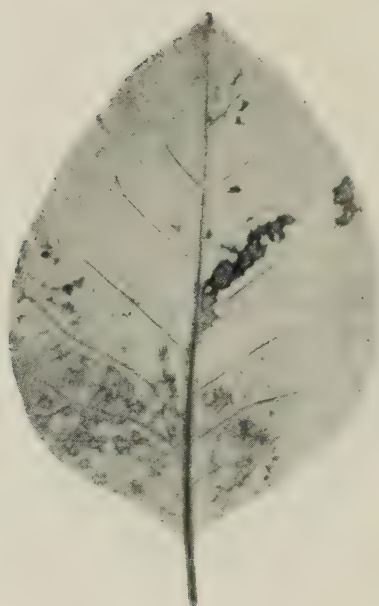


Fig. 3a



Fig. 3b

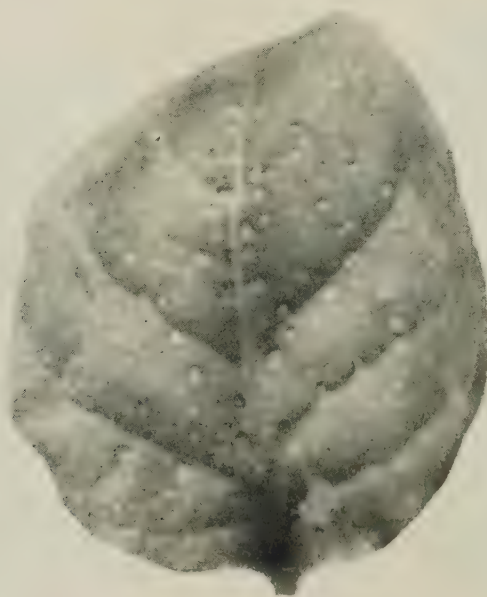


Fig. 4

Fig. 3. 5th day after inoculation. a = control, b = experiment.

Fig. 4. Necrotic spots on leaves of young *N. tabacum* Samsun plants after watering with cadmium sulphate solution (dose 320 mg. Cd^{2+} per plant). The spots resemble lesions on leaves of *N. glutinosa* after infection by TMV.

considerations which will be mentioned in the discussion, the experimental determination of the effect of cadmium ions on the reproduction of tobacco mosaic virus was undertaken. So far as is known to the author, the effect of this ion on the reproduction of plant viruses has not yet been studied.

Material and Methods

All experiments were carried out with tobacco plants (*Nicotiana tabacum* Samsun) at various stages of growth, cultivated in pots in the glasshouse.

Cadmium ions were applied in the form of sulphate. The experimental plants were watered with a solution of concentration 4 mg. Cd^{2+} in 1 ml. Doses of cadmium were tested within the range of 20 to 400 mg. Cd^{2+} to one plant. Watering with the experimental solution was carried out immediately and 24 hours before inoculation. In some of the experimental series plants were watered with the cadmium sulphate solution for a second time, on the third day following inoculation.

The Al-strain of tobacco mosaic virus (BLATTNÝ 1955) was used for inoculation because of its short incubation period. One leaf of each plant was inoculated, by the usual technique with carborundum. There were twenty plants in each variant of the experiment. The experiments were carried out from May to October 1958.

Following inoculation the course of primary infection was followed by means of the iodine test on starch (HOLMES 1931), in each variant on five leaves. The inoculated leaves were cut off, killed by plunging into boiling water, chlorophyll was extracted from them by boiling in 96% alcohol and they were then submerged in a dilute solution of iodine in potassium iodide.

On about the seventh day after inoculation, when the youngest leaves of the control plants began to show signs of systemic disease, the tips of the plants were cut off, homogenized and the relative virus concentration was determined by testing on *Nicotiana glutinosa* and on a hypersensitive hybrid¹⁾, which has been evolved by Květa Schwammenhöferová.

The content of free and labile phosphorus in tobacco leaves following watering with cadmium sulphate solution was determined orientationally. Phosphorus was determined by the modified method according to PONS and GUTHRIE (1946) and ZELLER (1949). Plant homogenates were deproteinized with 0.25 M trichloroacetic acid.

Results

Figures 1 to 3 show the differences in the course of primary infection between experimental and control plants from the June series. It is clear from the figures that cadmium ions delay the reproduction of tobacco mosaic virus. The following facts are particularly striking: in comparison with the control the leaves of experimental plants lose starch more slowly, the development of chlorotic spots is slower and their delimitation and structure are not sharp.

The results of quantitative tests on *N. glutinosa* and the hybrid with necrotic reaction are summarized in table 1. This table shows that the optimal dose of cadmium ions, which has no injurious effect on the plants themselves, is a dose of 160 mg. Cd^{2+} to one plant, applied in two waterings, one im-

¹⁾ Hybrid: Trapezond 161 $\times$ 8/37 (8/37—new selection VÚTP—Báb pri Sereďi). This material was used for the quantitative testing of TMV for the first time. It was subjected to preliminary testing and results compared with those from *N. glutinosa*.

Table 1.

Date of experiment	No. of developed leaves on exp. plants	Dose of Cd ²⁺ in mg. per plant	Watering with Cd ²⁺ solution before inoculation	Inhibition % ¹⁾	No. of lesions on half leaf of <i>N. glutinosa</i> or hypersensitive hybrid		P	n
					Control	Experiment		
21. 5.	8	60	immediately	45.0	183.1 ± 3. 25.7	102.4 ± 3. 10.6	10 ⁻²	8
27. 5.	8	80 + 80 ²⁾	immediately	71.5	63.8 ± 3. 11.4	18.2 ± 3. 4.1	10 ⁻⁴	18
11. 6.	8	80 + 80	immediately	69.8	49.2 ± 3. 9.2	14.7 ± 3. 2.4	10 ⁻⁴	18
13. 6.	8	130	immediately	61.2	32.5 ± 3. 5.2	12.6 ± 3. 2.2	10 ⁻⁴	24
13. 6.	12	130	immediately	55.3	27.7 ± 3. 5.8	12.3 ± 3. 2.5	10 ⁻²	19
15. 8.	9	80 + 80	immediately	64.0	107.6 ± 3. 14.7	38.7 ± 3. 5.4	10 ⁻⁵	25
15. 8.	9	80 + 80	24 hours	5.7	83.0 ± 3. 8.9	74.2 ± 3. 7.8	3. 10 ⁻¹⁴⁾	25
15. 8.	10	80 + 80	immediately	67.8	531.2 ± 3. 90.6	170.8 ± 3. 28.6	10 ⁻⁴	15
15. 8.	10	80 + 80	24 hours	46.9	353.8 ± 3. 91.4	187.6 ± 3. 50.5	10 ⁻¹⁴⁾	15
28. 9.	8	80 + 80	24 hours	19.4	270.2 ± 3. 24.7	217.7 ± 3. 19.6	10 ⁻¹⁴⁾	19
29. 9.	8	400 ³⁾	immediately	83.7	85.6 ± 3. 23.8	13.9 ± 3. 3.2	10 ⁻³	11
1. 10.	4	160 + 160	immediately	76.3	50.3 ± 3. 6.1	11.9 ± 3. 1.4	10 ⁻⁹	25

¹⁾ Calculated according to UTECH and JOHNSON (1950).²⁾ Second watering carried out in all series on the third day.³⁾ Concentrations inhibiting plant growth.⁴⁾ Not statistically significant. Statistical methods according to HRUBÝ (1950) were applied.

mediately before inoculation and the other on the third day after inoculation. Inhibition of tobacco mosaic averaged 70%. It is interesting that the inhibitory effect of cadmium ions was found to decrease considerably when the experimental plants were watered with cadmium sulphate solution 24 hours before inoculation.

Larger doses of cadmium ions, about 250 mg. to one plant, inhibit growth slightly. Naturally, a very important factor is the age of the plant. Following watering with cadmium ion solution containing a 320 mg. dose, necrotic spots (fig. 4) appear on the leaves of young tobacco plants (*N. tabacum* Samsun). These resemble the necrotic lesions on the leaves of *N. glutinosa* following inoculation with TMV.

The results also show that tobacco plants can stand a relatively high dose of cadmium without injury if the solution is applied by watering. The question of the amount of cadmium ions that get into the plant remains open.

When the leaves are sprayed necrosis appears on them even with concentrations of 1.5 μg . Cd^{2+} in 1 ml. This fact also did not permit direct proof to be given that inhibition of the virus in the inoculum is not involved. The results obtained while following the course of primary infection are regarded as sufficient in this respect (fig. 1 to 3). The number of chlorotic spots on the inoculated leaves of the experimental plants was the same as in the control.

In preliminary experiments changes in the content of inorganic and labile phosphorus in tobacco leaves following watering with cadmium sulphate solution were followed. Although the methodic aspect of these experiments is very difficult, because the age of the plants must be taken into account and watering does not allow of exact dosing with cadmium, these orientational experiments seem to indicate that the smaller doses of cadmium ions increase the content of inorganic phosphorus; on the contrary, larger doses, which delay plant growth, decrease the free phosphorus content to nearly one half. It will be possible to arrive at a final conclusion on this question only after a thorough analysis has been made.

Discussion

A number of investigations have been carried out on the effect of cadmium on higher plants (e. g. SCHARRE and SCHROPP 1934, SPENCER 1937, SCHARRE 1941). Most of these have been carried out from the point of view of plant nutrition, but none of them deals with the physiological effects of this element. All authors agree that cadmium ions are definitely more poisonous than zinc ions.

PAŘÍZEK (1956, 1957) found that cadmium is a very strong poison for male gonads, i. e. organs with a very intensive nucleoprotein biosynthesis and with relatively high zinc content. In the view of the above author, cadmium injures the testes because it excludes zinc, which is linked to nucleoprotein metabolism as a biogenic element. Following the application of cadmium, intensive decomposition of nucleotides takes place and phosphorus is freed and washed out of the bodies of the experimental animals.

Very little is known as yet about the biological function of zinc ions in plants (SCHARRER 1941, CHESTERS and ROLINSON 1951). The most typical symptoms of zinc deficiency are disturbances in chlorophyll synthesis and dwarf growth. Recently FUJII (1956) found that some cells, e. g. the staminal hair cells of *Tradescantia*, contain a relatively high content of zinc at the prophase. The last-mentioned facts would seem to indicate that zinc in plants is intimately connected with growth and cell division and that it is most probably involved in nucleoprotein metabolism, and therefore also in virus biosynthesis. This assumption is finally supported by the work of HELMS and POUND (1955), as mentioned in the introduction. They show the dependence of growth of the host and virus reproduction on dosing with zinc.

The results obtained so far from experiments on the inhibitory effect of cadmium ions on TMV reproduction do not permit the author to draw any conclusions regarding the mechanism of the inhibitory effect of these ions. One of the possible hypotheses, which might be adopted, is an analogy with the conception put forward in animal physiology that cadmium displaces zinc, which is linked with nucleoprotein metabolism. The alternative cannot, however, be excluded, i. e. that cadmium as a chemically related element can to a certain extent compensate for or take the place of zinc.

In conclusion it may be said that further study under more exact conditions of the already established inhibitory effect of cadmium ions on the reproduction of tobacco mosaic virus could contribute not only to a deeper understanding of the mechanism of the biosynthesis of virus nucleoproteins, but also to the explanation of the physiological effect of cadmium ions and of the biological function of zinc ions.

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Ионы кадмия — ингибитор вируса табачной мозаики

МАРИЕ УЛРЫХОВА-ЗЕЛИНКОВА

Резюме

Изучалось действие ионов кадмия на репродукцию вируса табачной мозаики на табаке (*Nicotiana tabacum* Samsun), выращиваемом в горшках в оранжерее. Подопытные растения поливались раствором сульфата кадмия концентрации 4 мг Cd^{2+} /1 мл в разных дозах. У каждого растения инокулирован один лист А1 штаммом ВТМ. Оптимальной дозой ионов кадмия, не имеющей отрицательное влияние на подопытные растения, является доза 160 мг Cd^{2+} на одно растение, примененная в двух поливах, как раз перед инокуляцией и на третий день после инокуляции. Репродукция ВТМ ингибирована из 70% по результатам теста на *N. glutinosa* и гиперсенситивном гибриде. Ингибирующее действие ионов кадмия значительно понижается в случае полива раствором кадмия 24 часа до инокуляции. Из различий хода первичной инфекции, изучаемых при помощи йодного теста, особенно знаменательны следующие факты: подопытные растения теряют из зараженных листьев крахмал медленнее, развитие хлоротических пятен заторможено и структуры не резкие. Завышенные дозы кадмия (около 300 мг на одно растение) вызывают на некоторых молодых растениях табака (*N. tabacum* Samsun) пятна, напоминающие некротические пятна на листьях *N. glutinosa* после заражения ВТМ. В качестве предварительных данных установлено, что ионы кадмия вызывают нарушения в метаболизме фосфора. В дискуссии формулирована гипотеза, что ингибирующее действие ионов кадмия на репродукцию ВТМ может базироваться — по аналогии с представлениями в физиологии животных — на взаимодействии ионов кадмия и цинка в метаболизме нуклеопротеинов.

The Effect of Extracted Humus Substances on Growth of Tomato Plants

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Souhrn

Jednotlivé složky humusu — alkoholický extrakt, huminové kyseliny a fulvokyseliny — postupně izolované z kompostové zahradní zeminy, zbavené elektrodialysou minerálních iontů, ukázaly po přidání do Sachsova živného roztoku rozdílnou aktivitu v působení na růst lodyhy a kořene rajčat. Obdobně bylo potvrzeno rozdílné ovlivnění příjmu kyslíku listy i kořeny a stanoveno různé množství chlorofylu v listech. Fulvokyseliny se ukázaly aktivnější složkou humusu než huminové kyseliny. Morfologické změny kořenového systému se ukázaly typičtější pro posouzení působnosti jednotlivých izolovaných složek humusu než uvedené změny ve fyziologických charakteristikách.

Summary

The different components of humus—alcoholic extract, humic acids and fulvic acids—progressively isolated from compost garden soil and deprived of mineral ions by electrodialysis, were, after addition to Sachs's nutrient solution, found to have differing effects on the growth of stems and roots of tomato plants. Similarly, differences in the effect on oxygen intake of leaves and roots was confirmed and varying amounts of chlorophyll were determined in the leaves. Fulvic acids were shown to be more active components of humus than humic acids. Morphological changes in the root system were found to be more typical for an estimation of the action of the different components of humus than the changes in physiological characteristics mentioned above.

Introduction

Papers dealing with the biological influence of humus on the plant (WAKSMAN 1936, KONONOVA 1951, PRÁT 1954) give evidence of its far-reaching effects on many physiological processes. Mention is made of changes in the various processes from chemotropic irritation, stimulation, changes in plasmatic structure, increased absorption of mineral ions, changes in the enzymatic system, increase in growth, intensification of synthetic processes and respiration, greater fertility and more rapid development. The explanation of such a wide range of effects must be sought above all in the influence of the whole complex of substances composing humus, with their varying biological activity depending on the origin of the material, the method of isolation and the sensitivity of the experimental object chosen. The data existing so far on the biological effects of humus on plants are mainly based on the study of the action of humus as a whole or of humic acids. Relatively little attention has been paid to other components such as fulvic acids, hymatomelanic acids and many others (NIKLEWSKI and WOJCIECHOWSKI 1937, KONONOVA and PANKOVA 1950, KHRISTEVA 1951 and TROJANOWSKI 1954).

In order that it may be possible to make a successful approach to the solution of important questions regarding the influence of the quality of humus on plant growth, increased stress is placed on the necessity of studying the action of isolated components of humus (NAJMR 1957) and of a thorough knowledge of their various modes of action (TROJANOWSKI 1952). No study of the complex action of humus components is, however, possible without a detailed knowledge of anatomical-morphological changes and of the consequences of changed physiological processes in the organism. It is remarkable that very little work has been devoted to the detailed study of the morphological effects of humus substances. And yet in plants, as in all living organisms, there exists a close connection between physiological processes and the anatomical-morphological formation of plant organs. It was necessary to compare the action of the different components of humus both on physiological processes and on morphological changes and to assess whether the effects of these substances are similar or diverse, and whether the chemical relationship between the humic acids and fulvic acids (MISTERSKI and LOGINOW 1957) evinces itself both physiologically and morphologically. Therefore, experiments were undertaken with Imun tomato plants grown in Sachs' nutrient solution with an optimal dose of extracted humus substances (see further). Observation of physiological changes was accompanied by the investigation of changes in morphological structure.

Material and Methods

Fractional extraction of compost garden soil was carried out and the following humus components were successively obtained:

1. Alcoholic extract
1. Humic acids
3. Fulvic acids

The soil was first extracted with 96% ethyl alcohol in the cold, using a total of 75 litres to 10 kg. of soil. The alcohol was then distilled under reduced pressure with an infra lamp (temperature 35° to 40° C). The residue of the extract was dissolved in distilled water at 50° C and electrodyalyzed by a 110 V current until the intensity dropped below 10 mA. The yellow solution thus obtained was filtered through a blue-banded paper filter, the filtrate being then used as alcoholic extract. The soil after being extracted with alcohol was dried at laboratory temperature, decalcified with 1% HCl, and humic acids were obtained by means of preparation according to FLAIG, SCHEFFER and KLAMROTH (1955). Fulvic acids were obtained by PONOMAREV's method (1947) of coagulating the acid filtrate. Both components, humic acids and fulvic acids, were electrodyalyzed in the same way as the alcoholic extract. The concentrations of the humus components were determined from the respective solutions by evaporation in a vacuum drier at 80° C. Humus substances remained in solution and were kept in a refrigerator at 0° to 5° C.

During preliminary experiments the optimal concentration of humus substances in the nutrient solution was established according to the growth of the tomato stems. The regular experimental series was started on March 30, 1958 in Sachs' nutrient solution with the addition of the appropriate amount of AZ solution. The tomatoes were precultivated in nutrient solution with the addition of humus substances in concentrations given below. At the phase of two assimilatory leaves (May 14) they were transferred to litre cultivation vessels and tied to a silon net. The following experimental series were set up:

1. Control plants growing only in Sachs' solution without humus substances.
2. Plants in Sachs' nutrient solution with the addition of humus substances of optimal effect:
 - a) 10 mg. of alcoholic extract to 1 litre
 - b) 50 mg. of humic acids to 1 litre
 - c) 50 mg. of fulvic acids to 1 litre

There were six cultivation vessels, each containing two plants, in each series of the experiment. In all series at the start of the experiment the pH of the nutrient solution was 6.5. Differences in the action of the humus substances were recorded after 14 weeks of cultivation (July 14). Changes in the height, length and weight of overground parts and roots were measured. Changes in oxygen intake were followed with a Warburg apparatus and the amount of chlorophyll was determined photocolorimetrically according to GODNEV (1952).

Results

The experiments showed that the selected humus components, when added to nutrient solution, have varying effects on the growth of tomato plants. This is first apparent in changes in physiological processes, in more intensive growth, and as a reflection of changed metabolic processes morphological differences in the overground parts and root system also arise. The values given have been statistically computed with the significance stated in table 1. It is evident from the results that the fulvic acids are most active in their effects on the plants. As compared with the control highly significant differences are to be found in the height of stems, fresh weight of stems and roots and the dry weight of stems of tomato plants grown in nutrient solution with added fulvic acids. Only the difference in length of the root system is not significant and in the dry weight of the roots is just significant.

A similar effect, although somewhat weaker, is to be observed with humic acids. Only the length of the root system is greater and the difference as compared with the control is highly significant. The alcoholic extract significantly increased the length of stems and root system, the fresh and dry weights of the roots and exhibited in general a less strong effect on the plants. It was found that the alcoholic fraction increased the amount of water both

Table 1. Dimensions and weight of Imun tomato plants growing in Sachs' nutrient solution with addition of humus components

		Control	Alcoholic extract	Humic acids	Fulvic acids
Height of stem in cm.		20.95 ± 3.4.14	32.44 ± 3.2.33 *	51.52 ± 3.2.23 **	56.76 ± 3.1.58 **
Length of roots in cm.		13.05 ± 3.0.94	16.99 ± 3.1.11 *	20.16 ± 3.1.11 **	14.05 ± 3.1.58
Fresh weight in g. (Gv)	stems	6.40 ± 3.1.50	8.85 ± 3.1.15	14.93 ± 3.0.52 **	17.48 ± 3.0.27 **
	roots	1.55 ± 3.0.38	3.29 ± 3.0.48 **	3.24 ± 3.0.51 **	5.39 ± 3.0.46 **
Dry weight in g. (Go)	stems	0.52 ± 3.0.12	0.62 ± 3.0.16 *	1.07 ± 3.0.12 *	1.60 ± 3.0.15 **
	roots	0.05 ± 3.0.01	0.09 ± 3.0.01 **	0.23 ± 3.0.07 *	0.24 ± 3.0.07 *
Gv/Go	stems	12.26	14.23	13.91	10.92
	roots	32.29	36.56	14.09	22.94

Difference * significant $P < 0.05$ when $P = 0.05$ $t = 2.06$
(24)

** highly significant $P < 0.01$ when $P = 0.01$ $t = 2.80$
(24)

in overground parts and roots, while humic acids caused an increase only in the stems. Fulvic acids reduced the amount of water in the whole plant. Changes in the oxygen intake of leaves and roots of tomato plants, as determined by the Warburg manometric technique, are recorded in table 2. It appeared that humus substances increase oxygen intake for respiration—in the case of overground parts fulvic acids are in the first place in this respect, followed by humic acids; in the case of roots the order is, alcoholic extract, fulvic acids, humic acids. Changes in chlorophyll content of leaves of equal age are given in table 3. They show that humus components, particularly fulvic acids and humic acids, also increase the amount of chlorophyll.

The action of the different humus components on the overground parts and particularly on the formation of the root system is characteristic. Figs. 1a to 1d show the overground parts of tomato plants from different experimental series. As compared with the control (1a) an increase in stem length and in the sizes of individual leaves can be observed progressively from alcoholic extract (1b), humic acids (1c) to fulvic acids (1d). Figs. 2a to 2d show the

Table 2. Oxygen intake in ml. by leaves and roots of Imun tomato plants per 1 mg. dry weight per hour

	Control	Alcoholic extract	Humic acids	Fulvic acids
Leaves	1.757	1.934	2.183	2.283
Increase compared with control		10%	24%	30%
Roots	0.172	0.302	0.212	0.237
Increase compared with control		76%	23%	38%

Table 3. Amount of chlorophyll in mg. in 1 g. of dry weight of leaves of Imun tomato plants in 1 litre solvent. Average of six repetitions

	Control	Alcoholic extract	Humic acids	Fulvic acids
Leaves	1.213	1.581	1.983	2.054
Increase compared with control		30%	63%	69%

differences in the formation of the root system in these experimental series and figs. 3a to 3d illustrate differences in the branching of primary roots in the same order. The stems of the control plants are weak at this period, with short internodes, and are the smallest (fig. 1a). The upper leaves are yellowish-green and the basal leaves fall. The root system is composed of white roots with swollen root tips (fig. 2a). Half way along the length of the primary roots isolated short secondary roots are to be found (fig. 3a). In the alcoholic extract the stems are longer with short internodes (fig. 1b). The leaves are clear green and basal leaves do not fall so readily. The root system is strongly developed (fig. 2b). The roots are long and pure white. Lateral roots grow along the whole length of the primary root and are long and branching (fig. 3b).

Tomato plants in humic acids form even longer stems with longer internodes (fig. 1c). The leaves are large, of a deep green colour, the basal leaves go yellow and fall. The roots are fine, richly branched and brown (fig. 2c). The long primary roots form fine lateral roots, mainly from the upper half; these branch further (fig. 3c). The root tips are often destroyed.

In fulvic acids the tomato plants form the longest stems and internodes (fig. 1d). The leaves are large, deep green and those at the base also turn yellow and fall. The root system in fulvic acids resembles that of the control in its general aspect, but the number of secondary roots is greater (fig. 2d). The primary roots are pure white, but much thicker than in the control and with more abundant short lateral roots, mainly on the lower half (fig. 3d).

Z. SLADKÝ

EXTRACTED HUMUS SUBSTANCES AND GROWTH



Fig. 1. Influence of humus substances on the growth of the overground parts of tomato plants in Sachs' nutrient solution: 1a control, 1b alcoholic extract 10 mg./l., 1c humic acids 50 mg./l., 1d fulvic acids 50 mg./l. Humus substances concentrations used are optimal.

EXTRACTED HUMUS SUBSTANCES AND GROWTH

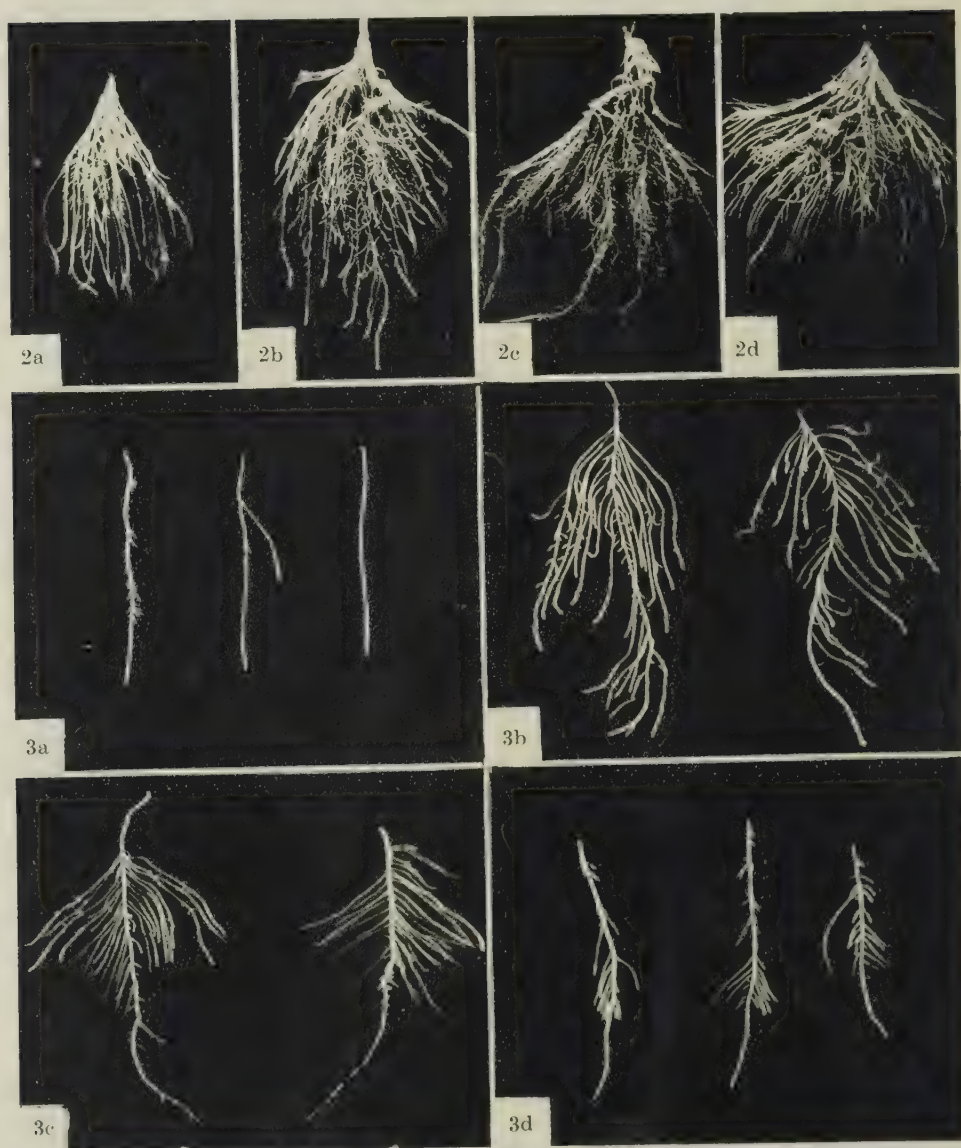


Fig. 2. Root system formation of tomato plants in Sachs' nutrient solution: 2a control, 2b alcoholic extract, 2c humic acids and 2d fulvic acids. Humus substances concentration the same as fig. 1.

Discussion

The great number of substances forming humus and their relationship to each other considerably complicates research not only on biochemical questions and the origin and composition of humus, but also on the question of its influence on the plant. Contemporary chemical methods of isolation are not adequate for the division of humus into its chemical components and therefore the term humus substances still covers a complex of substances of the most various chemical properties. The classification introduced by ODEN (1922), dividing humus substances on the basis of solubility into three groups, is not only of historical significance. It is still in general use and formed the basis for the isolation method of humus substances employed in the present experiments.

The results of these experiments demonstrate that the presence of humus substances in nutrient solutions causes a greater development of the root system so that the absorbent root surface is increased several times above normal. If we take into account the other changes in the plant which precede the increased root development, such as changes in the enzymatic system (SAALBACH 1956), in metabolism (TICHÝ 1958, PRÁT, ČATSKÝ and MELICHAR 1957), changes in plasma structure (NIKLEWSKI and WOJCIECHOWSKI 1937, GUMIŃSKI 1947), greater permeability of cell walls (LIESKE 1932, BŁOGOWESTSCHENSKI and PROSOROWSKAJA 1934, 1935, CHAMINADE and BLANCHET 1952), we see that by their complex action humus substances help the plant to increase intake of some mineral nutrients and are beneficial in accelerating the whole metabolism.

It is further evident from the results that humus substances induce increased intensity of respiration both in overground parts and roots. From the published work of OPARIN and GELMAN (1952) we have evidence of a close connection between increased respiration and increased synthesis. Other work (BANDURSKI 1957) points to an intimate connection between respiration, as a process releasing energy, and growth, which is typified as a process of energy accumulation with the co-operation of the enzymatic systems. The changes in oxygen intake, which have been followed for this purpose, provide us with a picture not only of respiration in the tissues, but also of the intensity of synthetic processes, the results of which are expressed in the values for growth recorded in table 1. Acceleration of growth, and increase in fresh weight and dry weight, the intensity of which is typical for the action of each component of humus, are in direct relation to the increase in consumption. There is an exception in the case of increased oxygen consumption by tomato roots in alcoholic extract, where this relation is not quite definite. In agreement with BIBER and MAGAZINER (1951) it was found that fulvic acids cause more intensive respiration than do humic acids, which undoubtedly is connected with a general influencing of the activity of respiratory ferments (KHRISTEVA 1953).

The amount of water in the stems and roots fluctuates considerably. The different extracted humus substances cause varying increases in dry weight of stems and roots and point to increased synthetic activity in the plant. This is most evident in the case of fulvic acids. The presence of humus sub-

stances in the nutrient solution produces an increase in and activation of the chlorophyll apparatus, which NIKLEWSKI and WOJCIECHOWSKI (1937) believe to be directly influenced by humus substances. Increased chlorophyll content was confirmed colorimetrically as in the work of PRÁT, ČATSKÝ and MELICHAR (1957) and the amount of chlorophyll was calculated according to GODNEV (1952). It was found that the amount of chlorophyll increased progressively from the series in alcoholic extract, to the humic acids and was highest with the fulvic acids. It is evident from these experiments that the fulvic acids are an even more effective component of humus than the humic acids and that the influence of alcoholic extract on the processes followed is less. The chemical relationship between humic acids and fulvic acids, established by physico-chemical study (MISTERSKI and LOGINOW 1957), is also clear from their physiological action. However, morphological changes, particularly of the root system, were found to be much more typical and reliable as indicators of the action of the different extracted humus components. The figures show clearly that humus substances encourage development of the root system, the growth of which is from the start typical for the different substances. The vitality of the whole plant is, of course, dependent on the growth and structure of the roots.

Alcoholic extract in the nutrient solution caused increase in length of the root system and abundant branching. The fresh weight of stems was increased by 38% and of roots by 112%, mainly due to increase in water content. The root system showed the highest oxygen consumption. This is connected not only with increased synthetic activity in the plant, but also with more intense activity of the pericambium in the formation of lateral roots.

The lengthening of roots was greatest in the humic acids. Fine, brown roots were formed. The fresh weight of the stems was increased by 133% and of the roots by 109%, mainly due to increased water content of the stems and transfer of assimilates to the roots, i. e. to a certain extent similarly to the process in alcoholic extract. Fulvic acids produced not only the greatest increase in fresh weight of stems (170%) and roots (245%) but also an increase in dry weight of 205% for stems and 390% for roots. The explanation is mainly to be sought in an increase of synthetic processes and in the chlorophyll content of the leaves. The strongly-developed root system was notable for the number of primary roots with relatively short lateral rootlets.

It is clear from the above that the different humus components exert varying influences on the metabolic processes of plants and effect their physiological processes with varying intensity. The action of the different humus components makes itself apparent in various forms in the general structure of the plant, in particular of the root system.

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Действие экстрагированных гумусовых веществ на рост томатов

ЗДЕНЕК СЛАДКÝ

Резюме

Отдельные составные части гумуса — алкогольный экстракт, гуминовые кислоты и фульвокислоты, поочередно изолированные из навозной огородной почвы и освобожденные путем электродиализа от минеральных ионов, показали после прибавления их в питательный раствор *Saxha* различную активность в своем действии на рост стебли и корни томата. Подтверждено также различное их влияние на прием кислорода листьями и корнями и определено различное содержание хлорофилла в листьях. Фульвокислоты оказались по сравнению с гуминовыми кислотами более активной частью гумуса. Морфологические изменения корневой системы оказались более типичными для обсуждения действия отдельных изолированных частей гумуса чем приведенные изменения в физиологических характеристиках.

Гамма-гексахлорциклогексан в качестве полиплоидогенного вещества

ЗДЕНЕК ЛАНДА

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Souhrn

Jedním z hlavních momentů šlechtitelského využití polyploidie je otázka polyploidisačních agens a metod jejich aplikace. Kromě nejužívanějšího alkaloidu kolchicinu je známa řada látek téhož účinku, z nichž některé mají dokonce ekonomické přednosti ve srovnání s kolchicinem, jsou však méně intenzivně studovány. Cílem této práce bylo ověřit údaje o polyploidisační aktivitě γ -hexachlorcyklohexanu, stanovit jednoduchou metodu jeho aplikace, zjistit šíři jeho působení a vyjasnit možnosti záměny kolchicinové techniky snáze dostupnou metodou aplikace γ -HCH. Metoda, která vyplynula z orientačních pokusů na cibuli a salátu, byla v dalším aplikována na druhy a odrůdy řady rostlin (viz seznam). Pokusy byla potvrzena polyploidisační aktivita γ -HCH. Perspektivnost použité modifikace výsevu semen do vlhkého filmu γ -HCH byla stanovena pro druhy *Linum usitatissimum*, *Viola tricolor maxima* a *Lactuca sativa*. Pro širší využití agens je třeba vypracovat příslušné modifikace. Zdá se, že bude možno užívat v cytologické technice místo kolchicinového pretreatment γ -HCH. V práci se pokračuje.

Резюме

Из методов, которые вызывают полиплоидию у растений, и которые являются одним из основных моментов ее селекционного использования, имеют прежде всего значение методы химические, в частности ряд модификаций, основанных на применении колхицина. Кроме колхицина известен ряд веществ, вызывающих полиплоидию. Некоторые из них имеют даже экономические преимущества по сравнению с колхицином, однако, их менее интенсивно изучают. Целью предлагаемой работы ориен-

тировочного характера ставилась проверка данных о полиплоидогенной активности гамма-гексахлорциклогексана (γ -ГХЦГ), установление простого метода его применения, систематического диапазона его действия и выяснение возможности замена колхицинового метода легче доступным применением γ -ГХЦГ. Предварительные опыты ставились на луковичах *Allium cepa* и на семенах *Lactuca sativa* с применением растворов агента в метаноле, этаноле и в воде. Вытекающий из них метод посева семян во влажный фильм γ -ГХЦГ применен в дальнейшем в серии опытов к семенному материалу ряда видов и сортов преимущественно культурных растений. Наблюдениями за габитусом роста и цитологическими отношениями подтверждена активность агента в вызывании полиплоидии. Перспективность использования примененного метода выявлена для видов *Lactuca sativa* L., *Viola tricolor maxima*, *Linum usitatissimum* L. Для более широкого использования агента следует выработать соответствующие модификации. Указано также на возможность использования γ -ГХЦГ в цитологической технике в качестве претритмента (pretreatment). Работа продолжается в намеченных направлениях.

Введение

В последние годы уделяется у нас также должное внимание явлению полиплоидии, проблематика которого в той или другой степени затрагивает ряд ботанических, зоологических и микробиологических дисциплин, и также смежных наук. Хотя и число работ, имеющих отношение к явлению полиплоидии, достигает в настоящее время уже многих тысяч (ср. EIGSTI 1947), все же остается ряд полностью не решенных проблем, для конкретизации которых, однако, требовалось бы специального обзора.

Возможности селекционного использования явления полиплоидии зависят от трех основных моментов: от характера исходного материала для полиплоидной селекции, от эффективности применяемых методов вызывания полиплоидии и от эффективности приемов селекции на полиплоидной базе. Из ряда известных методов, которыми вызывают полиплоидию, наиболее широко используются химические методы, основанные, в частности, на применении колхицина. Имеется, однако, целый ряд веществ, обладающих не менее выраженной активностью вызывать полиплоидию у растений (EIGSTI 1947, Шмук 1951), которые своей доступностью имеют даже преимущество по сравнению с колхицином. Однако, методы их использования разрабатываются гораздо менее интенсивно.

В литературе имеются указания на полиплоидогенную активность ГХЦГ и некоторых его изомеров, в частности, γ -изомера (NYBOM и KNUTSSON 1947, цит. по NAMEL 1947, KOSTOFF 1948, РАМАНУДЖАМ и ПАРТХАСАРАТХИ 1956, RAO и KUNDU 1949, цит. по Р. В. А., ШТУБЕЕ 1958).

Целью предлагаемой работы являлась проверка этих указаний. Своей задачей мы ставили установление простого метода применения агента, выяснение систематического диапазона его действия и также выяснение возможности замена колхицинового метода легче доступным применением γ -ГХЦГ.

Предварительные опыты

Предварительные опыты ставились на луковицах *Allium cepa* и на семенах *Lactuca sativa* „Mělnický král máje“ (сорт Мелницкий король мая). В опытах использованы насыщенные при комнатной температуре растворы γ -ГХЦГ в метаноле и этаноле, разбавленные водой до концентраций: 0,01%; 0,05%; 0,1%; 0,5%; 1%; 1,5%; 2%. Дальше использованы насыщенные при той же температуре растворы γ -ГХЦГ в воде в 3 модификациях — в случае *Allium cepa*: насыщенный раствор, насыщенный раствор с пленкой γ -ГХЦГ на поверхности, насыщенный раствор с пленкой и избытком агента на дне; в случае *Lactuca sativa*: насыщенный раствор, насыщенный раствор + опудривание семян агентом, насыщенный при температуре кипения раствор. Контролями служили культуры в воде и в метаноле и в этаноле, разбавленных до тех же концентраций как и в опытной серии. На положительный эффект воздействия γ -ГХЦГ судили по типичному с-туморному характеру роста, т. е. по появлению ланцетообразных утолщений корешков *Allium cepa* L. и задержке роста и утолщению ростков *Lactuca sativa* L. В случаях алкогольных растворов наиболее сильно выражен эффект в концентрации 0,1—1,5%. Однако, реакция на воздействие агентом резко выражена во 2-ой и 3-ей вариантах водных растворов как у *Allium cepa* L. так и у *Lactuca sativa* L. Из результатов предварительных опытов вытекало, что наиболее удачной является модификация с применением водного раствора, в частности, насыщенного при повышенной температуре. Целью дальнейших опытов являлось, следовательно, применение этой модификации к семенному материалу ряда видов и сортов, и выявление таким образом систематического диапазона действия γ -ГХЦГ и возможности использования ее в качестве метода получения полиплоидных растений.

Материал и методика опытов

В качестве подопытного материала использованы нами следующие виды и сорта растений: *Antirrhinum maius* L., *Dianthus barbatus* L., *Helianthus annuus* L., *Helichrysum monstrosum*, *Lactuca sativa* L. (сорта: Americký hnědý, Batavia, называемый также Blonde de Paris, Black Seeded Simpson, Böters Treib, Maikönig Freiland, Mělnický král máje I, II (двух провененций), Trotskopf Brauner), *L. perennis* L., *L. saligna* L., *L. serriola* TORN., *L. virosa* L., *Lathyrus articulatus* BRIQ., *L. clymenum* L., *L. odoratus* L. (смесь гибридов), *L. sativus* L. (типы: «пинерский», «сладкобный из СССР», «кормовой из СССР»), *Lycopersicum esculentum* MILL. сорта: Červené drobné, Hanácké rané, Ostravské nízké, Průhonický Universal, Vrbičanské nízké, Zlatá královna, Žluté drobné, *Linum usitatissimum* L., сорт: Novum, *Melandrium album* GARCKE, *Pisum sativum* L., сорт Heinrichův raný, *Solanum* sect. *Tuberarium* виды: *S. Antipovici* I, II, III, *S. boergeri* BUK., *S. chacoense*, *S. commersoni* DUN., *S. demissum* LINDL., *S. etuberosum* LINDL., *S. gibberulosum* BUK., *S. laplaticum* BUK., *S. longipedicellatum* BUK., *S. schicki* Juz. et BUK., *S. schreiteri* BUK., *S. tlazcalense* HAWK., *S. tuberosum* L., автогамия сорта Nowa Hula, *S. vernal* BITT. et WITTM., *Trifolium pratense* L., сорт Přerovský, *Viola tricolor maxima* сорта Berna и Záře Alp, *Vicia calcarata*, *V. cornigera*, *V. ervilia* WILD., *V. hybrida* L., *V. lathyroides* L., *V. ludoviciana*, *V. lutea* L., *Zea mays* L. — ряд сортов и гибридов.

Препарат γ -ГХЦГ (99—100%) получен из Энтомологической лаборатории ЧСАН.

На основании предварительных опытов применена нами следующая методика: около 0,25 гр. γ -ГХЦГ заливают $\frac{1}{2}$ л. воды, доводят до кипения, кипятят в течение 3—5 мин., затем раствор охлаждают, начинающий едва мутнеть вливают на фильтровальную бумагу в чашки Петри и оставляют на ночь. Утром устраняют избыточный раствор и в образовавшийся на бумаге фильм γ -ГХЦГ высевают семена. Время воздействия до нескольких дней.

Опыты ставились в 3 повторениях. Оценка полиплоидогенного действия γ -ГХЦГ проводилась наблюдением за с-эффектом роста (задержка, утолщение ростка, укорочение корней, вздутие их кончиков) и цитологическая проверка с-эффекта быстрыми цитологическими методами: нигрозиновым и ацетокарминовым (Нривѹ и Раѹовкѹвѹ 1952, Раѹовкѹвѹ и Раѹовек 1955). Этот способ выявления полиплоидогенной активности испытуемых веществ общепринят (ср. Шмук 1951).

Сводка результатов и обсуждение

Результаты опытов сведены в таблицу (табл. 1).

Табл. 1. Реакция видового материала на действие γ -ГХЦГ

Виды	Цитологический с-эффект	С-эффект габитуса	Пригодность исп. метода
<i>Antirrhinum majus</i>	+	++	—
<i>Dianthus barbatus</i>	+	+	—
<i>Helichrysum monstrosum</i>	+	—	—
<i>Helianthus annuus</i>	+	—	—
<i>Lactuca sativa</i>	+	++	+
<i>Lactuca</i> spp.	+	++	+
<i>Lathyrus odoratus</i>	+	+	—
<i>Lycopersicum esculentum</i>	+	+	—
<i>Linum usitatissimum</i>	+	++	+
<i>Melandrium album</i>	+	+	—
<i>Pisum sativum</i>	+	—	—
<i>Solanum</i> sect. <i>Tuberarium</i> spp.	+	+	—
<i>Viola tricolor maxima</i>	+	++	+
<i>Vicia</i> spp.	+	+	—
<i>Zea mays</i>	+	++	—
<i>Trifolium pratense</i>	+	+	—

Примечания к таблице 1 ст. 2: знаком + обозначены случаи с-митоза

ст. 3: знаком ++ обозначены случаи типичной реакции ростков или корешков, знаком + обозначено заметное проявление типичной реакции

ст. 4: пригодность метода оценивалась по хорошо выраженному с-эффекту ростков, не сопровождаемому повреждением корешка.

Следовательно, реакция на воздействие γ -ГХЦГ, оцениваемая по типу роста ростков, у разных видов различия. Во всех случаях, где мы имели дело с разными сортами, наблюдалась различная реакция. Действие γ -ГХЦГ вызывает типичные для полиплоидогенных веществ цитологи-

ческие реакции (резкое нарушение хода митоза, подавление веретена, разная степень полиплоидии, нарушения распределения хромосом). Детальный цитологический анализ действия γ -ГХЦГ, однако, не входил в задачи данной работы и ему будет посвящена отдельная работа. Следует все же отметить, что цитологические картины, наблюдавшиеся после воздействия агентом, дают основание для его применения в цитологической технике в качестве претритмента (pretreatment).

В связи с продолжающейся работой по использованию γ -ГХЦГ в качестве полиплоидогенного вещества следует обратить внимание на ряд вопросов. Прежде всего следует изучить характер распределения полиплоидных тканей в получаемых после воздействия миксоплоидных растениях и выяснить постоянность вызванной полиплоидии. Далее следует обратить внимание на подробное выяснение цитологической реакции всех изомеров ГХЦГ, что интересно в связи с теорией полиплоидогенного действия, на вопрос цитологического и физиологического действия в практике сельского хозяйства применяемых доз агента. В литературе также подробно не выяснены конкретные причины различий в реакции отдельных видов и сортов. Актуальным является также вопрос селективной стимуляции развития полиплоидных тканей. Работа продолжается в намеченных нами направлениях.

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Hexachlorocyclohexane as a Polyploidisation Agent

ZDENĚK LANDA

Summary

We have confirmed data regarding the action of γ -hexachlorocyclohexane as an agent inducing polyploidy, established a simple method of its application, determined the extend of its effect and found out whether it would be possible to replace the colchicine technique by a more accesible method of γ -HCH application. The method resulting from preliminary experiments with onion and lettuce was later applied to species and varieties of a number of plants (see list). The activity of γ -HCH as an agent inducing polyploidy was confirmed by the experiments. A method of sowing seeds in a damp film of γ -HCH was projected for *Linum usitatissimum*, *Viola tricolor maxima* a *Lactuca sativa*. It seems probable that the use of γ -HCH instead colchicine pretreatment will be possible in cytological technique.

Über den Einfluss äthanollöslicher organischer Kompoststoffe auf den Zuckermetabolismus in grünen Pflanzenteilen

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Souhrn

V předložené práci byl studován vliv ethanolicke frakce půdneho humusu na rovnováhu glycidů v izolovaných listech pšenice (*Triticum vulgare* VILL.), lípy (*Tilia cordata* MILL.) a jírovce (*Aesculus hippocastanum* L.).

Bylo zjištěno, že humusové látky rozpustné v ethanolu způsobují snížení obsahu neredukujících cukrů v listech pokusných rostlin a zvýšení výdeje kyslíku uhlíkatého. Poněvadž výdej CO_2 není úměrný úbytku neredukujících cukrů ani všech cukrů, je třeba předpokládat, že ethanolicke frakce podporuje i jiné přeměny neredukujících cukrů, které mají za následek jejich úbytek v listech. Citlivým měřítkem vlivu ethanolicke frakce je velikost poměru N : R (poměr neredukujících cukrů k redukujícím), jehož hodnota je snižována.

Srovnávací pokus s humátem draselným ukázal, že tato frakce působí na rostlinu jinak; snižuje obsah redukujících cukrů, zatím co množství cukrů neredukujících je spíše zvyšováno. Dýchání není po 8 hod. pokusu podstatně změněno.

Ethanolicke frakce i humát draselný značně zvyšují příjem glukosy z roztoku listovými řapíky.

Zusammenfassung

In der vorliegenden Arbeit ist der Einfluss der äthanolischen Fraktion des Bodenumus auf das Gleichgewicht der Glycide in isolierten Blättern von Weizen (*Triticum vulgare* VILL.) Linde (*Tilia cordata* MILL.) und Rosskastanie (*Aesculus hippocastanum* L.) studiert worden.

Es wurde festgestellt, dass die äthanollöslichen Humusstoffe eine Herabsetzung des Gehaltes an nicht reduzierenden Zuckern in den Blättern der

Versuchspflanzen und eine Erhöhung der Kohlendioxydausscheidung bewirken. Weil die CO_2 -Ausscheidung weder dem Abnehmen der nicht reduzierenden, noch überhaupt aller Zucker entspricht, ist es notwendig vorauszusetzen, dass die äthanolische Fraktion auch andere Umsätze der nicht reduzierenden Zucker unterstützt, welche deren Abnahme in der Blättern bewirken. Ein empfindlicher Indikator des Einflusses der äthanolischen Fraktion ist die Grösse des Verhältnisses N : R (des Verhältnisses der nicht reduzierenden zu den reduzierenden Zuckern), dessen Wert herabgesetzt erscheint.

Ein Versuch mit Kaliumhumat zu Vergleichszwecken zeigte, dass diese Fraktion auf die Pflanzen anders wirkt. Sie setzt den Inhalt der reduzierenden Zucker herab, während die Menge der nicht reduzierenden Zucker eher erhöht wird. Die Atmung erscheint nach 8 Stunden des Versuches nicht wesentlich verändert.

Die äthanolische Fraktion sowie Kaliumhumat erhöhen die Aufnahme von Glukose aus der Lösung durch die Blattstiele der Pflanzen stark.

Einleitung

In meiner vorhergehenden Arbeit habe ich die Wirkung der Alkohol-Benzen-Fraktion des Bodenumus, der Huminsäuren und der Fulvosäuren aufs Gleichgewicht der reduzierenden und nicht reduzierenden Zucker in den oberirdischen Teilen des Weizens beschrieben (TICHÝ 1959). Von den untersuchten Stoffen wirkte die Alkohol-Benzen-Fraktion in der Richtung einer Ansammlung von reduzierenden Zuckern in den Weizenblättern zu Lasten der nicht reduzierenden Zuckern. Im Charakter ihrer Wirkungsweise gemahnt also die Alkohol-Benzen-Fraktion an den Einfluss von Thymohydrochinon auf den Inhalt von reduzierenden Zuckern und Stärke in Getreidepflänzchen, von dem FLAIG, SCHARRE und SCHOLL (1957a) berichteten. In ihren Versuchen kam es unter der Wirkung des Thymohydrochinons zur Ansammlung reduzierender Zucker und zur Herabsetzung des Stärkegehaltes in jungen Hafer- und Kornpflänzchen. An anderer Stelle zeigten die gleichen Autoren (FLAIG, SCHARRE und SCHOLL 1957b), dass das Thymohydrochinon auch die Aktivität einiger Pflanzen-Enzyme, z. B. der Aldolase, Amylase, Saccharase und Phosphatase in keimenden Kornpflänzchen beeinflusste. Ausserdem stellten FLAIG und SAALBACH (1956) fest, dass Thymohydrochinon auch die Atmung junger Korn- und Weizenpflänzchen beeinflusst (resp. die Aktivität der Dehydrasen erhöht).

Im Hinblick auf ihre experimentellen Ergebnisse und zahlreiche literarische Angaben finden FLAIG, SCHARRE und SCHOLL (1957a) eine gewisse Analogie zwischen der Wirkung des Thymohydrochinons und einigen Wachstums-Stimulatoren und nähern sich darin weiter der Erklärung des Zusammenhanges zwischen den physiologischen, den Wachstums- und den formenden Einflüssen dieses Stoffes, den sie für ein Modell der Humusstoffe halten.

Es ist ohne Zweifel, dass sich manche Wachstums-Stimulatoren auch im Boden und insbesondere in Komposten vorfinden können (ROBERTS u. ROBERTS 1939; STEWART und ANDERSON 1942; KONONOVA 1951; LINSER 1955).

welche zum Einfluss auf den Wuchs und zum Metabolismus der Pflanzen beitragen können. Im Hinblick auf ihre gute Äthanollöslichkeit kann man dann erwarten, dass sie sich gerade an den Einflüssen der Alkohol-Benzen-Fraktion beteiligen werden.

Die vorhergehende Arbeit (TICHÝ 1959), in der ich eine gewisse Ähnlichkeit zwischen der Wirkung des Thymohydrochinons und der Alkohol-Benzen-Fraktion des Komposthumus festgestellt habe, weist darauf hin. Aufgabe dieser Arbeit ist es, die Kenntnisse der Einflüsse der Alkohol-Benzen-, resp. der Äthanolauzüge der Komposte auf den Metabolismus der Glycide in den oberirdischen Teilen der Pflanzen zu vertiefen und sie insbesondere mit Rücksicht auf ihren Zusammenhang mit der Pflanzenatmung zu entwickeln.

Material und Methode

Für die Versuche habe ich junge Blätter des Frühjahrsweizens „Niva“ (*Triticum vulgare* VILL.) der Linde (*Tilia cordata* MILL.) und der Rosskastanie (*Aesculus hippocastanum* L.) verwendet. Die untersuchten Fraktionen habe ich aus Komposterde gewonnen, die einem vier Jahre alten Kompost entnommen war, welcher aus Blattabfällen zubereitet worden ist. Ich extrahierte diese Komposterde mit einer Mischung von Äthanol und Benzen (1 : 1) in einer Soxhlet-Apparatur, den Extrakt dampfte ich ein und den Rückstand zog ich mit einer minimalen Menge Äthanol in der Wärme aus. Eine so gewonnene Fraktion bezeichne ich im weiteren als äthanolische Fraktion. Nach der Zugabe von Wasser zum Äthanolkonzentrat entstand eine Suspension, aus der ich den Äthanolrest auf dem Wasserbade mit Unterdruck abdampfte. Mit der Bestimmung des Eindampfdruckstandes habe ich die Konzentration der wässrigen Suspension festgestellt, die ich nach gründlicher Verdünnung in meinen Versuchen anwandte. Diese Suspension habe ich in einer Menge, die 10 mg Abdampfdruckstand enthielt, einem Liter Wasser oder Zuckerlösung hinzugefügt, in die dann die Blätter der Versuchspflanzen mit ihren Stielen eingetaucht wurden. In diesen Blättern habe ich im Verlaufe des Versuches, resp. nach seiner Beendung den Gehalt an reduzierenden und nicht reduzierenden Zuckern mittels der Methode nach BERTRAND bestimmt.

Die Versuche habe ich in drei Abschnitte eingeteilt; im ersten Abschnitt wurden zwei Varianten durchgeführt. Bei einer von ihnen habe ich Lindenblätter verwendet, bei der zweiten Rosskastanienblätter. Diese wurden am 23. V. 1956 abgeschnitten. Etwa ein Viertel habe ich rasch getrocknet und für die Zuckerbestimmung verwendet. Die restlichen drei Viertel habe ich 24 Stunden lang mit ihren Stengeln in 6% Saccharose eingetaucht. Dann wurden die Blätter herausgenommen und in drei gleiche Teile geteilt. Der erste wurde zum Zwecke der Zuckerbestimmung getrocknet. Der zweite wurde mit seinen Stengeln in Leitungswasser eingetaucht und der letzte in eine Suspension aus alkoholischer Fraktion. Nach weiteren 24 Stunden habe ich auch diese Blätter getrocknet und ihren Zuckergehalt bestimmt.

Im zweiten Abschnitte der Versuche wurden Blätter 10 Tage alter Weizenpflänzchen verwendet, gezüchtet auf Sandkulturen, die mit Wasser begossen wurden. Die abgeschnittenen Blätter wurden zu je 50 Stück mit ihrer Schnittfläche in folgende Lösungen gelegt:

A — Wasser (Kontrolle), B — 6% Glukoselösung, C — 6% Glukoselösung mit einer Zugabe von 10 mg/L alkoholischer Fraktion, D — 6% Glukoselösung mit einer Zugabe von 10 mg/L Kaliumhumat (Herstellungsweise siehe TICHÝ, 1958). In diesen Lösungen wurden die Pflanzen acht Stunden lang in diffusem Tageslichte gelassen, dann wurden sie herausgenommen, an ihrem unteren Teile abgespült, rasch getrocknet und es wurde ihr Zuckergehalt bestimmt.

Den dritten Versuch habe ich ähnlich wie den zweiten angelegt. Die Weizenblätter wurden: A — in Wasser gelegt (Kontrolle), B — in eine wässrige Suspension eines Äthanolauzuges der angeführten Humus-Kompost-Stoffe in einer Konzentration von 10 mg/L und C — in eine Kaliumhumatlösung von gleicher Konzentration. Zugleich wurde bei den einzelnen Varianten die Menge des ausgeatmeten Kohlendioxyds in einer in Abb. 1 veranschaulichten Apparatur gemessen. Der erste Versuch hatte eine Dauer von acht Stunden und nach seiner Beendung wurden die Blätter wiederum aus der Lösung genommen, getrocknet und ihr Zuckergehalt wurde ebenso wie in den vorhergegangenen Versuchen bestimmt.

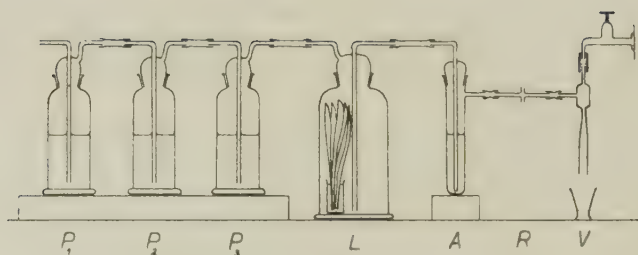


Abb. 1. Apparatur zur Bestimmung der ausgeatmeten Kohlendioxydmenge. Eine Wasser-Saugpumpe (V) saugt Luft aus der Apparatur, die nacheinander durch zwei Waschflaschen mit konzentrierter Kalilauge (P_1 und P_2) geleitet wird und durch eine Kontrollflasche (P_3) mit $\text{Ba}(\text{OH})_2$ strömt. Von hier steigt sie in den oberen Teil der Flasche (L), in welcher sich ein kleines Gefäß Flüssigkeit befindet, in die die Blätter der Versuchspflanze mit ihren Basen eingetaucht sind. Vom Boden der Flasche (L) wird die Luft in das Absorptionsgefäß (A) abgesaugt, in welchem der Kohlendioxyd von $\text{Ba}(\text{OH})_2$ — Lösung aufgenommen wird. Die beschriebene Einrichtung stellt eine Arbeitseinheit dar. Beim Versuch wurden immer sechs solcher Einheiten mittels eines Verteilers (R) an eine Wassersaugpumpe angeschlossen.

Ergebnisse

Die Ergebnisse des ersten Versuches sind aus Abb. 2 und 3 ersichtlich. In den Blättern der Linde stieg in der Saccharoselösung der Gehalt an nicht reduzierenden Zuckern von 5,76 auf 6,89%, also um 19,6%. Der Gehalt

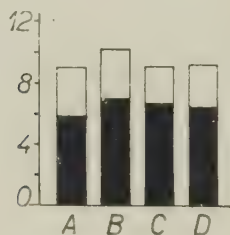


Abb. 2.

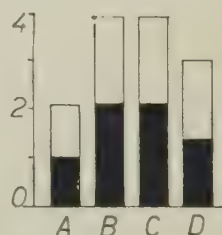


Abb. 3.

Abb. 2 und 3. Inhalt der reduzierenden Zucker (leere Kolonnen), nicht reduzierenden Zucker (schwarze Kolonnen) der Lindenblätter (Abb. 2.) und Rosskastanienblätter (Abb. 3.) vor dem Versuch (A), nach Sättigung mit Saccharose (B) und nach folgender Behandlung mit Wasser (C) oder äthanolischer Fraktion (D). Achse y —%.

reduzierender Zucker hatte sich nicht verändert. In den Rosskastanienblättern erhöhte sich unter gleichen Bedingungen der Gehalt an nicht reduzierenden Zuckern von 1,01 auf 2,15%, also um 114%, der Gehalt an reduzierenden Zuckern von 1,07 auf 1,80%, also um 68%. In so präparierten Lindenblättern

sank nach dem Eintauchen in Wasser der Gehalt beider Zuckerfraktionen (der Gehalt an reduzierenden Zuckern um 22%, an nicht reduzierenden um 6%). In Rosskastanienblättern hat er sich nicht verändert. Sowie die Blätter jedoch der Einwirkung der äthanolischen Fraktion ausgesetzt wurden, sank

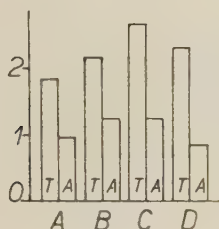


Abb. 4. Verhältnis der nicht reduzierenden zu den reduzierenden Zuckern (N : R) von Lindenblättern (Kolonne T), Rosskastanienblättern (Kolonne A) vor dem Versuche (A), nach Sättigung mit Saccharose (B) und folgender Behandlung mit Wasser (C) oder äthanolischer Fraktion (D). Achse y — Verhältnis N : R.

der Gehalt an nicht reduzierenden Zuckern bei Linde um 8%, in Rosskastanienblättern um 35%. Gleichzeitig sank der Gehalt an reduzierenden Zuckern in Lindenblättern um 13% und in Rosskastanienblättern um 9%.

Beim zweiten Versuch (Abb. 5) stieg bei Weizenblättern, die in Glukoselösung eingetaucht worden waren, der Gehalt an reduzierenden Zuckern von

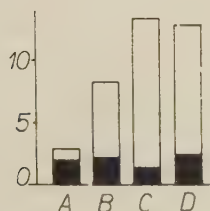


Abb. 5. Inhalt der reduzierenden Zucker (leere Kolonnen) und nicht reduzierenden Zucker (schwarze Kolonnen) von Weizenblättern, die mit ihren Basen eingetaucht wurden: A — in Wasser, B — in Glukoselösung, C — in Glukoselösung mit äthanolischer Fraktionsbeigabe und D — in Glukoselösung mit Kaliumhumatbeigabe. Achse y — %.

0,81 auf 6,15%. Das Ansteigen des Gehaltes an nicht reduzierenden Zuckern war demgegenüber weit schwächer, nämlich von 2,05 auf 2,24%, also betrug es 10%. Wenn zur Glukoselösung äthanolische Fraktion zugegeben worden war, äusserte sich ihr Einfluss durch ein weiteres, wesentlich vergrößertes Ansteigen von Glukose, deren Gehalt (reduzierende Zucker) auf 12,5% stieg, also um 110% im Vergleiche zu Pflanzen aus Glukoselösung allein. Gleichzeitig trat aber ein deutliches Absinken der nicht reduzierenden Zuckerfraktion ein und zwar um 36% gegenüber Blättern aus reiner Glukoselösung und um 30% gegenüber Blättern aus klarem Wasser. Der zu Vergleichszwecken durchgeführte Versuch mit Kaliumhumat zeigte — beim Vergleiche mit der Wirksamkeit reiner Glukose — eine geringfügige Erhöhung der Glukose-Aufnahme, die nur wenig höher ist, als die durch die äthanolische Fraktion bewirkte Erhöhung.

Der dritte Versuch (Abb. 6.) bestätigte, dass es in Weizenblättern, deren Basen in eine Suspension aus äthanolischer Fraktion eingetaucht wurden,

bei praktisch unveränderter Glukosemenge zu einer bedeutenden Herabsetzung des Saccharosegehaltes (um 30%) kommt. Gleichzeitig erhöht sich die Kohlendioxydausscheidung und zwar um 13%. Ein Vergleich mit dem Einflusse von Kaliumhumat zeigte, dass diese Fraktion unter gleichen Bedin-

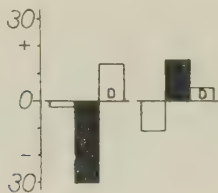


Abb. 6. Erhöhung (+%) und Herabsetzung (—%) des Gehaltes an reduzierenden Zuckern (leere Kolonnen) und nicht reduzierenden Zuckern (schwarze Kolonnen) von Weizenblättern, sowie die Menge des von ihnen ausgeatmeten Kohlendioxyds (Kolonne D) unter Einfluss der äthanolischen Fraktion (linkes Diagramm) und Kaliumhumat (rechtes Diagramm).

gungen den Inhalt der reduzierenden Zucker um 11% herabsetzt, während die Veränderungen des Gehaltes an nicht reduzierenden Zuckern im Vergleich zu ihrem absoluten Werte vernachlässigbar von 0,75 auf 0,86% der Trockensubstanz steigen. Ebenso war die Erhöhung der Kohlendioxydausscheidung geringfügig.

Diskussion

Die in dieser Arbeit beschriebenen Versuche haben gezeigt, dass Humusstoffe, die äthanollöslich sind, in den Metabolismus der Glycide der grünen Pflanzenteile in der Weise eingreifen, dass sie den Gehalt an nicht reduzierenden Zuckern herabsetzen, was sich in der Abnahme des Verhältniswertes der nicht reduzierenden Zucker zu den reduzierenden deutlich äussert. So bestätigen sie selbst bei Anwendung einer anderen Methode und an weiteren Pflanzen die angeführten Ergebnisse der vorhergegangenen Mitteilung (TICHÝ 1959).

Die Veränderungen des Zuckergehaltes in Lindenblättern sind nur unbedeutend und wenig ausdrucksvoll. Es hängt dies wahrscheinlich mit dem besonderen Charakter des Glycidemetabolismus der Linde zusammen, der sich in einem starken Pektinstoffgehalt äussert, der auch in einer Verschleimung der Lösungen zutage tritt, in denen sich Lindenblätter im Verlaufe der Versuche befanden. Demgegenüber ist die Herabsetzung der nicht reduzierenden Zucker bei Rosskastanie sehr ausdrücklich und auf Abb. 3. auf den ersten Blick zu erkennen. Der Charakter des Einflusses der alkoholischen Fraktion ist jedoch bei beiden Pflanzenarten deutlich erkennbar, wenn wir die gleichzeitigen Veränderungen beider Zuckerfraktionen beachten und ihr gegenseitiges Verhältnis $N : R$, d. h. das Verhältnis der nicht reduzierenden zu den reduzierenden Zuckern ausdrücken. Die Veränderungen dieses Verhältnisses sind in Abb. 4. dargestellt. Die zwei letzten Kolonnen zeigen im Vergleiche mit den vorletzten klar ein gleich ausdrucksvolles Absinken des Verhältnisses $N : R$ bei Linde und auch bei Rosskastanie.

Die vorhergehende Sättigung der Blätter mit Saccharose, die einen höheren Spiegel der nicht reduzierenden Fraktion und damit erweisbarere Unterschiede ihres Inhaltes sicherte, konnte allerdings auch die Versuchsergebnisse beeinflussen. Nichtsdestoweniger bestätigte auch ein weiterer Versuch, verbunden mit einer Applikation von Äthanolfraktion zugleich mit Glukose die Ergebnisse des ersten Versuches. Auch hier bewirkte die äthanolische Fraktion eine deutliche Herabsetzung des Gehaltes an nicht reduzierenden Zuckern (Abb. 5.), auch wenn die Blätter gleichzeitig eine grosse Menge Glukose aufgenommen hatten. Kaliumhumat hat diese Veränderungen nicht bewirkt, es führte eher zu einer geringfügigen Erhöhung des Gehaltes an nicht reduzierenden Zuckern. Bei den einzelnen Varianten veränderte sich das Verhältnis N : R folgendermassen:

Wasser	2,53
Glukose	0,36
Glukose und äthanolische Fraktion	0,12
Glukose und Kaliumhumat	0,23

Bei der Auswertung dieser Unterschiede darf man allerdings nicht vergessen, dass es unter dem Einfluss der Humusstoffe zu einer unmittelbaren Erhöhung der Glukoseaufnahme kam und zwar so, dass sie bei einer Erhöhung des Glukosegehaltes von 0,81 auf 6,12% bei Pflanzen aus Glukoselösung unter dem Einfluss von äthanolischer Fraktion auf 12,05% und von Kaliumhumat auf 10,67% stieg. Es ist bemerkenswert, dass diese Erhöhung bei Kaliumhumat niedriger liegt, als bei der äthanolischen Fraktion.

Zu einer Abnahme der nicht reduzierenden Zucker und mit ihr verbunden zu einem Absinken des Wertes N : R kann es auf verschiedene Weise kommen. Einmal ist es möglich, Ueberlegungen über eine Inhibition ihrer Synthese oder über eine Erhöhung ihrer Hydrolyse (wie FLAIG, SCHARER und SCHOLL 1957 a vermuten) anzustellen, dann kann dies aber auch durch eine Erhöhung der CO_2 -Abgabe bewirkt sein, wenn wir Saccharose, bzw. ihren Fruktoseteil als Kohlendioxydquelle ansehen. Nach Lvov (1952) kann in einem solchen Falle die Herabsetzung des Saccharosegehaltes von einem Ansteigen des reduzierenden Zuckerspiegels begleitet sein und zwar um das Äquivalent der Glukose, die durch eine Oxydation des Fruktoserestes freigeworden ist. Der dritte Versuch zeigt in der Tat, dass ein Absinken nicht reduzierender Zucker, welches durch die äthanolische Fraktion bewirkt worden ist, von einem Ansteigen der CO_2 -Produktion begleitet wird. Das stimmt gut mit den Angaben von CHRISTEVA (1953) überein, welche einen erhöhten Sauerstoffverbrauch bei Geweben fand, auf die sie mit der bituminösen Humusfraktion eingewirkt hatte. Die in dieser Arbeit beschriebenen Versuche bestätigen jedoch einen ähnlichen Erfolg bei Kaliumhumat nicht, denn von den Veränderungen, die durch diesen Stoff hervorgerufen worden sind, ist nur das Absinken des Glukosegehaltes signifikant. Man muss sich allerdings gegenwärtigen, dass das Kaliumhumat in diesem Falle nur acht Stunden einwirken konnte, was schon im Hinblick darauf eine verm. kurze Frist ist, dass manche Autoren eine gewisse Induktionsperiode für die Wirkung

von Huminsäuren voraussetzen. (z. B. BLAGOWESTSCHENSKI und PROSOROWSKAJA 1935, GUMIŃSKI 1950). Beim letzten Versuch traten die Veränderungen des Verhältnisses N : R folgendermassen in Erscheinung:

Wasser	0,12,
äthanolische Fraktion	0,08,
Kaliumhumat	0,15.

Alle drei Versuche zeigen in Übereinstimmung mit der vorhergehenden Publikation (TICHÝ 1959), dass alkohollösliche Humusstoffe auf den Glycide-metabolismus einen spezifischen Einfluss ausüben, der in der Form eines Absinken des Gehaltes an nicht reduzierenden Zuckern in oberirdischen Pflanzenteilen, gegebenenfalls auch durch eine Erhöhung des Gehaltes reduzierender Zucker in Erscheinung tritt. Das gleichzeitige Absinken des Gehaltes an nicht reduzierenden Zuckern und das Ansteigen der CO_2 -Produktion, die im dritten Versuche festgestellt worden sind, charakterisieren die äthanolische Fraktion als ein Stimulans des Katabolismus, das im ganzen nicht zur Bildung von Trockensubstanz beiträgt, wie aus einer anderen Arbeit des Autors hervorgeht (TICHÝ 1958). Dabei machen sich zweifellos auch andere Zersetzungsprozesse geltend, denn die Ausscheidung von Kohlenstoff in der Form von CO_2 stellt nur 68% der Kohlenstoffabnahme aller Zucker dar. Das weist darauf hin, dass unter der Wirkung der äthanolischen Fraktion der Gehalt der nicht reduzierenden Zucker durch die Intensivierung auch anderer, als nur der Respirationsvorgänge abnimmt. In dieser Richtung muss man alle die Weisen nachprüfen, in denen diese Fraktion das Gleichgewicht zwischen den einzelnen Zuckergruppen beeinflusst.

Die Vergleichsvarianten mit Kaliumhumat zeigten, dass sich dieser Humusstoff hinsichtlich seiner Einwirkung auf den Glycide-Metabolismus anders verhält, als die äthanolische Fraktion und zwar in dem Sinne, dass er kein Absinken, sondern eher eine Erhöhung der nicht reduzierenden Zucker bewirkt, die man allerdings vorderhand nicht als beweiskräftig ansehen kann. Die Versuche zeigten, dass durch diese Einwirkung ein Absinken der reduzierenden Zucker bewirkt wird.

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О влиянии в этаноле растворимых гумусовых веществ на метаболизм сахаров в зеленых частях растений

ВЛАДИМИР ТИХЫ

Резюме

В предлагаемой работе изучалось влияние этанолной фракции почвенного гумуса на равновесие глицидов в изолированных листьях пшеницы (*Triticum vulgare* Vill.), липы (*Tilia cordata* Mill.) и каштана (*Aesculus hippocastanum* L.). Установлено, что в этаноле растворимые вещества приводят к снижению содержания нередуцирующих сахаров в листьях опытных растений и на повышение отдачи углекислого газа. Так как отдача CO_2 не пропорциональна снижению содержания нередуцирующих сахаров, следует полагать, что этанолная фракция способствует и другим переменам нередуцирующих сахаров, что влечет за собою падение их содержания в листьях. Весьма чувствительным мерилom влияния этанолной фракции является величина отношения $\text{N} : \text{R}$ (нередуцирующих к редуцирующим сахарам), значение которого снижается.

Сравнительный опыт с гуматом калия показал, что эта фракция действует на растение иначе; снижает содержание редуцирующих сахаров, между тем что количество нередуцирующих сахаров повышается. Дыхание в течение 8 часов опыта существенно не изменяется.

Этанолная фракция и гумат калия значительно повышают прием глюкозы из раствора черешками листьев.

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